

ANTITHROMBOTIC AGENTS**FIELD OF THE INVENTION**

5 This invention relates to monoclonal antibodies (mAbs) that bind to a human coagulation factor or cofactor and their use as self-limiting inhibitors of thrombosis in combination with plasminogen activators.

BACKGROUND OF THE INVENTION

10 Under normal circumstances, an injury, be it minor or major, to vascular endothelial cells lining a blood vessel triggers a hemostatic response through a sequence of events commonly referred to as the coagulation "cascade." The cascade culminates in the conversion of
15 soluble fibrinogen to insoluble fibrin which, together with platelets, forms a localized clot or thrombus which prevents extravasation of blood components. Wound healing can then occur followed by clot dissolution and restoration of blood vessel integrity and flow.

20 The events which occur between injury and clot formation are a carefully regulated and linked series of reactions. In brief, a number of plasma coagulation proteins in inactive proenzyme forms and cofactors circulate in the blood. Active enzyme complexes are
25 assembled at an injury site and are sequentially activated to serine proteases, with each successive serine protease catalyzing the subsequent proenzyme to protease activation. This enzymatic cascade results in each step magnifying the effect of the succeeding step.
30 For an overview of the coagulation cascade see the first chapter of "Thombosis and Hemorrhage", J. Loscalzo and A. Schafer, eds., Blackwell Scientific Publications, Oxford, England (1994).

35 While efficient clotting limits the loss of blood at an injury site, inappropriate formation of thrombi in veins or arteries is a common cause of disability and

death. Abnormal clotting activity can result in and/or from pathologies or treatments such as myocardial infarction, unstable angina, atrial fibrillation, stroke, renal damage, percutaneous transluminal coronary angioplasty, disseminated intravascular coagulation, sepsis, pulmonary embolism and deep vein thrombosis. The formation of clots on foreign surfaces of artificial organs, shunts and prostheses such as artificial heart valves is also problematic.

Approved anticoagulant agents currently used in treatment of these pathologies and other thrombotic and embolic disorders include the sulfated heteropolysaccharides heparin and low molecular weight (LMW) heparin. These agents are administered parenterally and can cause rapid and complete inhibition of clotting by activation of the thrombin inhibitor, antithrombin III and inactivation of all of the clotting factors.

However, due to their potency, heparin and LMW heparin suffer drawbacks. Uncontrolled bleeding as a result of the simple stresses of motion and accompanying contacts with physical objects or at surgical sites is the major complication and is observed in 1 to 7% of patients receiving continuous infusion and in 8 to 14% of patients given intermittent bolus doses. To minimize this risk, samples are continuously drawn to enable ex vivo clotting times to be continuously monitored, which contributes substantially to the cost of therapy and the patient's inconvenience.

Further, the therapeutic target range to achieve the desired level of efficacy without placing the patient at risk for bleeding is narrow. The therapeutic range is approximately 1 to less than 3 ug heparin/ml plasma which results in activated partial thromboplastin time (aPTT) assay times of about 35 to about 100 seconds. Increasing the heparin concentration to 3

ug/ml exceeds the target range and at concentrations greater than 4 ug/ml, clotting activity is not detectable. Thus, great care must be taken to keep the patient's plasma concentrations within the therapeutic range.

Another approved anticoagulant with slower and longer lasting effect is warfarin, a coumarin derivative. Warfarin acts by competing with Vitamin K dependent post-translational modification of prothrombin and other Vitamin K-dependent clotting factors. The general pattern of anticoagulant action, in which blood is rendered non-clottable at concentrations only slightly higher than the therapeutic range is seen for warfarin as well as for heparin and LMW heparin.

In acute myocardial infarction (MI), the major objectives of thrombolytic therapy include early and sustained reperfusion of the infarcted vessel. Present therapy for acute MI includes both a plasminogen activator, such as tissue plasminogen activator (tPA) or streptokinase and an anticoagulant such as unfractionated heparin, low molecular weight heparin or direct thrombin inhibitors or antiplatelet agents such as aspirin or platelet glycoprotein IIb/IIIa blocker. See Topol, *Am Heart J*, 136, S66-S68 (1998). This combination of therapies is based on the observation that clot formation and dissolution are dynamic processes and thrombin activity and generation continue after the formation of the occlusive thrombus and during and after dissolution of the clot. See Granger et al, *J Am Coll Cardiol*, 31, 497-505 (1998).

The optimal strategy for treatment of acute MI remains elusive and available agents and treatment protocols display both negative and positive characteristics. For example, fibrin-bound thrombin is insensitive to inhibition by heparin (Becker et al. in Chapter 6 of "Chemistry and Biology of Serpins", Plenum

Press, New York (1997)) and thrombin activity exhibits a rebound increase following cessation of heparin therapy with an observed increase in reinfarction within 24 hours following discontinuation of heparin. See Watkins
5 *et al.*, *Catheterization and Cardiovascular Diagnosis*, 44, 257-264 (1998) and Granger, *Circulation*, 91, 1929-1935 (1995). Further, antiplatelet agents may be accompanied by bleeding or thrombocytopenia.

Also, numerous clinical trials have shown that high
10 doses of thrombolytic agents lead to significant alteration in plasma hemostatic markers. See Rao *et al.*, *J Clin Invest*, 101, 10-14 (1988); Bovill *et al.*, *Ann Int Med*, 115, 256-265 (1991); Neuhaus *et al.*, *J Am Coll Cardiol*, 19, 885-891 (1992). Although increasing
15 concentrations of tPA lead to enhanced clot dissolution, the alteration in these hemostatic markers mirrors increased liabilities of thrombolytic therapy, particularly the incidence of severe bleeding.

Clearly, a need exists for antithrombotic agents
20 efficacious in controlling thrombotic disorders while maintaining hemostatic functions.

SUMMARY OF THE INVENTION

Accordingly, one aspect of the present invention is a method for inhibiting thrombosis in an animal
25 comprising administering an effective dose of an anti-coagulation factor monoclonal antibody having self-limiting neutralizing activity in combination with a plasminogen activator.

Another aspect of the invention is a method of
30 reducing a required dose of a thrombolytic agent in treatment of thrombosis in an animal comprising administering an anticoagulant specifically targeting a component of the intrinsic coagulation pathway in combination with the thrombolytic agent.

35 Another aspect of the invention is a method for reducing a required dose of a thrombolytic agent in

treatment of thrombosis in an animal comprising administering an anti-Factor IX monoclonal antibody in combination with the thrombolytic agent

5 **BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 is a graph of experimental results demonstrating the titration of normal human plasma with the murine anti-Factor IX mAbs BC1 and BC2.

Figure 2 is a graph of experimental results
10 demonstrating the titration of normal human plasma with the murine anti-Factor IX mAbs 9E4(2)F4 and 11G4(1)B9.

Figure 3 is a graph of experimental results demonstrating the titration of normal human plasma with the murine anti-Factor X mAbs HFXHC and HFXLC and the
15 murine anti-Factor XI mAb HFXI.

Figure 4 is a histogram of experimental results demonstrating the effect of heparin, acetylsalicylic acid and murine Factor IX mabs on activated partial thromboplastin time (aPTT) at 60 minutes in a rat
20 carotid thrombosis model.

Figure 5 is a histogram of experimental results demonstrating the effect of heparin, acetylsalicylic acid and murine Factor IX mabs on prothrombin time at 60 minutes in a rat carotid thrombosis model.

25 Figure 6 is a histogram of experimental results demonstrating the effect of heparin, acetylsalicylic acid and murine Factor IX mabs on occlusion of carotid artery flow in a rat carotid thrombosis model.

Figure 7 is a histogram of experimental results
30 demonstrating the effect of heparin, acetylsalicylic acid and murine Factor IX mabs on thrombus weight in a rat carotid thrombosis model.

Figure 8 is a histogram of experimental results demonstrating the effect of heparin, the murine Factor
35 IX mab BC2, a chimeric Factor IX mab and humanized

factor IX mAbs on aPTT at 60 minutes in a rat carotid thrombosis model.

Figure 9 is a histogram of experimental results demonstrating the effect of heparin, the murine Factor IX mab BC2, a chimeric Factor IX mab and humanized factor IX mAbs on thrombus weight in a rat carotid thrombosis model.

Figure 10 is a histogram of experimental results demonstrating the effect of anti-Factor IX mab and heparin on tPA-mediated reperfusion.

Figure 11 is a histogram of experimental results demonstrating the effect of anti-Factor IX mab and heparin on carotid vessel patency.

Figure 12 is a histogram of experimental results demonstrating the effect of anti-Factor IX mab and heparin on time to restoration of blood flow.

Figure 13 demonstrates the effect of tPA on the hemostatic parameters, fibrinogen, plasminogen and antiplasmin.

Figure 14 demonstrates the effect of tPA, heparin and anti-Factor IX mab on aPTT.

DETAILED DESCRIPTION OF THE INVENTION

All publications, including but not limited to patents and patent applications, cited in the specification are herein incorporated by reference as though fully set forth.

The present invention provides a variety of antibodies, altered antibodies and fragments thereof directed against coagulation factors, which are characterized by self-limiting neutralizing activity. Preferably, the coagulation factor is from the intrinsic or common coagulation pathway. Most preferably, the anti-coagulation factor antibodies are anti-Factor IX, anti-Factor IXa, anti-Factor X, anti-Factor Xa, anti-Factor XI, anti-Factor XIa, anti-Factor VIII, anti-

Factor VIIIIa, anti-Factor V, anti-Factor Va, anti-Factor VII, anti-Factor VIIa, anti-thrombin or anti-prothrombin. Particularly preferred are anti-Factor IX antibodies. Exemplary anti-coagulation factor

5 antibodies are the humanized monoclonal antibodies SB 249413, SB 249415, SB 249416, SB 249417, SB 257731 and SB 257732 directed against human Factor IX, the chimeric monoclonal antibody ch α FIX directed against human Factor IX, the murine monoclonal antibodies BC1, BC2, 9E4(2)F4

10 and 11G4(1)B9 which are directed against human Factor IX and/or Factor IXa or the murine monoclonal antibodies HFXLC and HFXI which are directed against human Factors X and XI, respectively. Particularly preferred is the anti-human Factor IX monoclonal antibody SB 249417.

15 The antibodies of the present invention can be prepared by conventional hybridoma techniques, phage display combinatorial libraries, immunoglobulin chain shuffling and humanization techniques to generate novel self-limiting neutralizing antibodies. Also included

20 are fully human mAbs having self-limiting neutralizing activity. These products are useful in therapeutic and pharmaceutical compositions for thrombotic and embolic disorders associated with myocardial infarction, unstable angina, atrial fibrillation, stroke, renal

25 damage, pulmonary embolism, deep vein thrombosis, percutaneous transluminal coronary angioplasty, disseminated intravascular coagulation, sepsis, artificial organs, shunts or prostheses.

As used herein, the term "self-limiting

30 neutralizing activity" refers to the activity of an antibody that binds to a human coagulation factor, preferably from the intrinsic and common pathways, including Factor IX/IXa, X/Xa, XI/XIa, VIII/VIIIIa and V/Va, VII/VIIa and thrombin/prothrombin and inhibits

35 thrombosis in a manner such that limited modulation of coagulation is produced. "Limited modulation of

coagulation" is defined as an increase in clotting time, as measured by prolongation of the activated partial thromboplastin time (aPTT), where plasma remains clottable with aPTT reaching a maximal value despite increasing concentrations of monoclonal antibody. This limited modulation of coagulation is in contrast to plasma being rendered unclottable and exhibiting an infinite aPTT in the presence of increasing concentrations of heparin. Preferably, the maximal aPTT value of the methods of the invention are within the heparin therapeutic range. Most preferably, maximal aPTT is within the range of about 35 seconds to about 100 seconds which corresponds to about 1.5 times to about 3.5 times the normal control aPTT value. In one embodiment of the invention, aPTT is prolonged without significant prolongation of prothrombin time (PT).

The phrase "in combination with" refers to administration of one therapeutic agent before, after or concurrent with the administration of another therapeutic agent in a single course of treatment.

"Altered antibody" refers to a protein encoded by an altered immunoglobulin coding region, which may be obtained by expression in a selected host cell. Such altered antibodies are engineered antibodies (e.g., chimeric or humanized antibodies) or antibody fragments lacking all or part of an immunoglobulin constant region, e.g., Fv, Fab, Fab' or F(ab')₂ and the like.

"Altered immunoglobulin coding region" refers to a nucleic acid sequence encoding an altered antibody of the invention. When the altered antibody is a CDR-grafted or humanized antibody, the sequences that encode the complementarity determining regions (CDRs) from a non-human immunoglobulin are inserted into a first immunoglobulin partner comprising human variable framework sequences. Optionally, the first

immunoglobulin partner is operatively linked to a second immunoglobulin partner.

"First immunoglobulin partner" refers to a nucleic acid sequence encoding a human framework or human immunoglobulin variable region in which the native (or naturally-occurring) CDR-encoding regions are replaced by the CDR-encoding regions of a donor antibody. The human variable region can be an immunoglobulin heavy chain, a light chain (or both chains), an analog or functional fragments thereof. Such CDR regions, located within the variable region of antibodies (immunoglobulins) can be determined by known methods in the art. For example Kabat et al. in "Sequences of Proteins of Immunological Interest", 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987) disclose rules for locating CDRs. In addition, computer programs are known which are useful for identifying CDR regions/structures.

"Second immunoglobulin partner" refers to another nucleotide sequence encoding a protein or peptide to which the first immunoglobulin partner is fused in frame or by means of an optional conventional linker sequence (*i.e.*, operatively linked). Preferably, it is an immunoglobulin gene. The second immunoglobulin partner may include a nucleic acid sequence encoding the entire constant region for the same (*i.e.*, homologous, where the first and second altered antibodies are derived from the same source) or an additional (*i.e.*, heterologous) antibody of interest. It may be an immunoglobulin heavy chain or light chain (or both chains as part of a single polypeptide). The second immunoglobulin partner is not limited to a particular immunoglobulin class or isotype. In addition, the second immunoglobulin partner may comprise part of an immunoglobulin constant region, such as found in a Fab, or F(ab)₂ (*i.e.*, a discrete part of an appropriate human constant region or framework

region). Such second immunoglobulin partner may also comprise a sequence encoding an integral membrane protein exposed on the outer surface of a host cell, e.g., as part of a phage display library, or a sequence
5 encoding a protein for analytical or diagnostic detection, e.g., horseradish peroxidase, β -galactosidase, etc.

The terms Fv, Fc, Fd, Fab, Fab' or F(ab')₂ are used with their standard meanings. See, e.g., Harlow et al.
10 in "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory, (1988).

As used herein, an "engineered antibody" describes a type of altered antibody, i.e., a full-length synthetic antibody (e.g., a chimeric or humanized
15 antibody as opposed to an antibody fragment) in which a portion of the light and/or heavy chain variable domains of a selected acceptor antibody are replaced by analogous parts from one or more donor antibodies which have specificity for the selected epitope. For example,
20 such molecules may include antibodies characterized by a humanized heavy chain associated with an unmodified light chain (or chimeric light chain), or vice versa. Engineered antibodies may also be characterized by alteration of the nucleic acid sequences encoding the
25 acceptor antibody light and/or heavy variable domain framework regions in order to retain donor antibody binding specificity. These antibodies can comprise replacement of one or more CDRs (preferably all) from the acceptor antibody with CDRs from a donor antibody
30 described herein.

A "chimeric antibody" refers to a type of engineered antibody which contains a naturally-occurring variable region (light chain and heavy chains) derived from a donor antibody in association with light and
35 heavy chain constant regions derived from an acceptor antibody.

A "humanized antibody" refers to a type of engineered antibody having its CDRs derived from a non-human donor immunoglobulin, the remaining immunoglobulin-derived parts of the molecule being
5 derived from one or more human immunoglobulins. In addition, framework support residues may be altered to preserve binding affinity. See, e.g., Queen et al., *Proc Natl Acad Sci USA*, 86, 10029-10032 (1989), Hodgson et al., *Bio/Technology*, 9, 421 (1991).

10 The term "donor antibody" refers to a monoclonal or recombinant antibody which contributes the nucleic acid sequences of its variable regions, CDRs or other functional fragments or analogs thereof to a first immunoglobulin partner, so as to provide the altered
15 immunoglobulin coding region and resulting expressed altered antibody with the antigenic specificity and neutralizing activity characteristic of the donor antibody. One donor antibody suitable for use in this invention is a murine self-limiting neutralizing
20 monoclonal antibody designated as BC2. Other suitable donor antibodies include the murine self-limiting neutralizing monoclonal antibodies designated as BC1, 9E4(2)F4, 11G4(1)B9, HFXLC and HFXI.

The term "acceptor antibody" refers to monoclonal
25 or recombinant antibodies heterologous to the donor antibody, which contributes all, or a portion, of the nucleic acid sequences encoding its heavy and/or light chain framework regions and/or its heavy and/or light chain constant regions to the first immunoglobulin
30 partner. Preferably, a human antibody is the acceptor antibody.

"CDRs" are defined as the complementarity determining region amino acid sequences of an antibody which are the hypervariable regions of immunoglobulin
35 heavy and light chains. See, e.g., Kabat et al., "Sequences of Proteins of Immunological Interest", 4th

Ed., U.S. Department of Health and Human Services,
National Institutes of Health (1987). There are three
heavy chain and three light chain CDRs or CDR regions in
the variable portion of an immunoglobulin. Thus, "CDRs"
5 as used herein refers to all three heavy chain CDRs, or
all three light chain CDRs or both all heavy and all
light chain CDRs, if appropriate.

CDRs provide the majority of contact residues for
the binding of the antibody to the antigen or epitope.
10 CDRs of interest in this invention are derived from
donor antibody variable heavy and light chain sequences,
and include analogs of the naturally occurring CDRs,
which analogs also share or retain the same antigen
binding specificity and/or neutralizing ability as the
15 donor antibody from which they were derived.

By "sharing the antigen binding specificity or
neutralizing ability" is meant, for example, that
although mAb BC2 may be characterized by a certain level
of self-limiting neutralizing activity, a CDR encoded by
20 a nucleic acid sequence of BC2 in an appropriate
structural environment may have a lower, or higher
activity. It is expected that CDRs of BC2 in such
environments will nevertheless recognize the same
epitope(s) as BC2.

25 A "functional fragment" is a partial heavy or light
chain variable sequence (e.g., minor deletions at the
amino or carboxy terminus of the immunoglobulin variable
region) which retains the same antigen binding
specificity and/or neutralizing ability as the antibody
30 from which the fragment was derived.

An "analog" is an amino acid sequence modified by
at least one amino acid, wherein said modification can
be chemical or a substitution or a rearrangement of a
few amino acids (i.e., no more than 10), which
35 modification permits the amino acid sequence to retain
the biological characteristics, e.g., antigen

specificity and high affinity, of the unmodified sequence. Exemplary analogs include silent mutations which can be constructed, via substitutions, to create certain endonuclease restriction sites within or
5 surrounding CDR-encoding regions.

Analogues may also arise as allelic variations. An "allelic variation or modification" is an alteration in the nucleic acid sequence encoding the amino acid or peptide sequences of the invention. Such variations or
10 modifications may be due to degeneracy in the genetic code or may be deliberately engineered to provide desired characteristics. These variations or modifications may or may not result in alterations in any encoded amino acid sequence.

The term "effector agents" refers to non-protein carrier molecules to which the altered antibodies, and/or natural or synthetic light or heavy chains of the donor antibody or other fragments of the donor antibody may be associated by conventional means. Such non-
20 protein carriers can include conventional carriers used in the diagnostic field, e.g., polystyrene or other plastic beads, polysaccharides, e.g., as used in the BIAcore (Pharmacia) system, or other non-protein substances useful in the medical field and safe for
25 administration to humans and animals. Other effector agents may include a macrocycle, for chelating a heavy metal atom or radioisotopes. Such effector agents may also be useful to increase the half-life of the altered antibodies, e.g., polyethylene glycol.

For use in constructing the antibodies, altered antibodies and fragments of this invention, a non-human species such as bovine, ovine, monkey, chicken, rodent (e.g., murine and rat) may be employed to generate a desirable immunoglobulin upon presentment with a human
35 coagulation factor, preferably factor IX/IXa, X/Xa, XI/XIa, VIII/VIIIa, V/Va, VII/VIIa or

thrombin/prothrombin or a peptide epitope therefrom. Conventional hybridoma techniques are employed to provide a hybridoma cell line secreting a non-human mAb to the respective coagulation factor. Such hybridomas
5 are then screened for binding using Factor IX/IXa, X/Xa, XI/XIa, VIII/VIIIa, V/Va, VII/VIIa or thrombin/prothrombin coated to 96-well plates, as described in the Examples section, or alternatively with biotinylated Factor IX/IXa, X/Xa, XI/XIa, VIII/VIIIa,
10 V/Va, VII/VIIa or thrombin/prothrombin bound to a streptavidin-coated plate. Alternatively, fully human mAbs can be generated by techniques known to those skilled in the art and used in this invention.

One exemplary, self-limiting neutralizing mAb of
15 this invention is mAb BC2, a murine antibody which can be used for the development of a chimeric or humanized molecule. The BC2 mAb is characterized by a self-limiting inhibitory activity on clotting time. As measured by the aPTT assay, the effect of the BC2 mAb on
20 clot time exhibits a maximal value of about 100 seconds. The BC2 mAb also binds Factor IXa, inhibits Factor IX to IXa conversion and inhibits Factor IXa activity. Divalent metal cofactors are required for activity, with the mAb exhibiting a greater preference for Ca^{2+} over
25 Mn^{2+} . The observed IC_{50} in the aPTT assay is approximately 50 nM. The BC2 mAb exhibits a species cross-reactivity with rat and is of isotype IgG2a.

Other desirable donor antibodies are the murine mAbs, BC1, 9E4(2)F4 and 11G4(1)B9. These mAbs are
30 characterized by a self-limiting inhibitory activity on clotting time. As measured by the aPTT assay, the effect of these mAbs on clot time exhibits a maximal value of about 90 to 100 seconds for 9E4(2)F4 and about 80 seconds for 11G4(1)B9. The BC1 mAb also binds Factor
35 IXa, inhibits Factor IXa activity but does not inhibit Factor IX to IXa conversion. A metal cofactor is not

required for its activity. The observed IC_{50} for BC1 in the aPTT assay is approximately 35 nM. The BC1 mAb is of isotype IgG1.

Yet another desirable donor antibody characterized by a self-limiting inhibitory activity on clotting time is the murine mAb HFXLC. As measured by the aPTT assay, the effect of the HFXLC mAb on clot time exhibits a maximal value of about 50 to 60 seconds. The HFXLC mAb binds Factor X light chain, and inhibits Factor X/Xa activity. The observed IC_{50} in the aPTT assay is approximately 20 nM.

Yet another desirable donor antibody characterized by a self-limiting inhibitory activity on clotting time is the murine mAb, HFXI. As measured by the aPTT assay, the effect of the HFXI mAb on clot time exhibits a maximal value of about 100 seconds. The HFXLC mAb binds Factor XI and inhibits Factor XI/XIa activity. The observed IC_{50} in the aPTT assay is approximately 30 nM.

While not intending to be bound to any particular theory regarding the mechanism of action, these mAbs appear to regulate coagulation by a non-competitive or allosteric mechanism whereby only partial inhibition is achieved.

This invention is not limited to the use of the BC1, BC2, 9E4(2)F4, 11G4(1)B9, HFXLC, HFXI or their hypervariable (*i.e.*, CDR) sequences. Any other appropriate high-affinity antibodies characterized by a self-limiting neutralizing activity and corresponding CDRs may be substituted therefor. Identification of the donor antibody in the following description as BC1, BC2, 9E4(2)F4, 11G4(1)B9, HFXLC or HFXI is made for illustration and simplicity of description only.

The present invention also includes the use of Fab fragments or $F(ab')_2$ fragments derived from mAbs directed against the appropriate human coagulation factor or cofactor. These fragments are useful as

agents having self-limiting neutralizing activity against coagulation factors, preferably against Factor IX/IXa, X/Xa, XI/XIa, VIII/VIIIa, V/Va, VII/VIIa or thrombin/prothrombin. A Fab fragment contains the entire light chain and amino terminal portion of the heavy chain. An F(ab')₂ fragment is the fragment formed by two Fab fragments bound by disulfide bonds. The mAbs BC1, BC2, 9E4(2)F4, 11G4(1)B9, HFXLC and HFXI and other similar high affinity antibodies, provide sources of Fab fragments and F(ab')₂ fragments which can be obtained by conventional means, e.g., cleavage of the mAb with the appropriate proteolytic enzymes, papain and/or pepsin, or by recombinant methods. These Fab and F(ab')₂ fragments are useful themselves as therapeutic, prophylactic or diagnostic agents, and as donors of sequences including the variable regions and CDR sequences useful in the formation of recombinant or humanized antibodies as described herein.

The Fab and F(ab')₂ fragments can be constructed via a combinatorial phage library (see, e.g., Winter et al., *Ann Rev Immunol*, 12,433-455 (1994)) or via immunoglobulin chain shuffling (see, e.g., Marks et al., *Bio/Technology*, 10, 779-783 (1992), which are both hereby incorporated by reference in their entirety, wherein the Fd or v_H immunoglobulin from a selected antibody (e.g., BC2) is allowed to associate with a repertoire of light chain immunoglobulins, v_L (or v_K), to form novel Fabs. Conversely, the light chain immunoglobulin from a selected antibody may be allowed to associate with a repertoire of heavy chain immunoglobulins, v_H (or Fd), to form novel Fabs. Self-limiting neutralizing Factor IX Fabs can be obtained by allowing the Fd of mAb BC2 to associate with a repertoire of light chain immunoglobulins. Hence, one is able to recover neutralizing Fabs with unique

sequences (nucleotide and amino acid) from the chain shuffling technique.

The mAb BC2 or other antibodies described above may contribute sequences, such as variable heavy and/or
5 light chain peptide sequences, framework sequences, CDR sequences, functional fragments, and analogs thereof, and the nucleic acid sequences encoding them, useful in designing and obtaining various altered antibodies which are characterized by the antigen binding specificity of
10 the donor antibody.

The nucleic acid sequences of this invention, or fragments thereof, encoding the variable light chain and heavy chain peptide sequences are also useful for mutagenic introduction of specific changes within the
15 nucleic acid sequences encoding the CDRs or framework regions, and for incorporation of the resulting modified or fusion nucleic acid sequence into a plasmid for expression. For example, silent substitutions in the nucleotide sequence of the framework and CDR-encoding
20 regions can be used to create restriction enzyme sites which facilitate insertion of mutagenized CDR and/or framework regions. These CDR-encoding regions can be used in the construction of the humanized antibodies of the invention.

25 The nucleic and amino acid sequences of the BC2 heavy chain variable region are listed in SEQ ID NOs: 5 and 7. The CDR sequences from this region are listed in SEQ ID NOs: 8, 9 and 10.

The nucleic and amino acid sequences of the BC2
30 light chain variable region are listed in SEQ ID NOs: 6 and 11. The CDR sequences from this region are listed in SEQ ID NOs: 12, 13 and 14.

Taking into account the degeneracy of the genetic code, various coding sequences may be constructed which
35 encode the variable heavy and light chain amino acid sequences and CDR sequences of the invention as well as

functional fragments and analogs thereof which share the antigen specificity of the donor antibody. The isolated nucleic acid sequences of this invention, or fragments thereof, encoding the variable chain peptide sequences
5 or CDRs can be used to produce altered antibodies, e.g., chimeric or humanized antibodies or other engineered antibodies of this invention when operatively combined with a second immunoglobulin partner.

It should be noted that in addition to isolated
10 nucleic acid sequences encoding portions of the altered antibody and antibodies described herein, other such nucleic acid sequences are encompassed by the present invention, such as those complementary to the native CDR-encoding sequences or complementary to the modified
15 human framework regions surrounding the CDR-encoding regions. Useful DNA sequences include those sequences which hybridize under stringent hybridization conditions to the DNA sequences. See, T. Maniatis et al., "Molecular Cloning: A Laboratory Manual", Cold Spring
20 Harbor Laboratory (1982), pp. 387-389. An example of one such stringent hybridization condition is hybridization at 4XSSC at 65°C, followed by a washing in 0.1XSSC at 65°C for one hour. Alternatively, an exemplary stringent hybridization condition is 50%
25 formamide, 4XSSC at 42°C. Preferably, these hybridizing DNA sequences are at least about 18 nucleotides in length, i.e., about the size of a CDR.

Altered immunoglobulin molecules can encode altered antibodies which include engineered antibodies such as
30 chimeric antibodies and humanized antibodies. A desired altered immunoglobulin coding region contains CDR-encoding regions that encode peptides having the antigen specificity of a Factor IX/IXa, X/Xa, XI/XIa, VIII/VIIIa, V/Va, VII/VIIa or thrombin/prothrombin
35 antibody, preferably a high affinity antibody such as provided by the present invention, inserted into a first

immunoglobulin partner such as a human framework or human immunoglobulin variable region.

Preferably, the first immunoglobulin partner is operatively linked to a second immunoglobulin partner.

5 The second immunoglobulin partner is defined above, and may include a sequence encoding a second antibody region of interest, for example an Fc region. Second immunoglobulin partners may also include sequences encoding another immunoglobulin to which the light or
10 heavy chain constant region is fused in frame or by means of a linker sequence. Engineered antibodies directed against functional fragments or analogs of coagulation factors may be designed to elicit enhanced binding with the same antibody.

15 The second immunoglobulin partner may also be associated with effector agents as defined above, including non-protein carrier molecules, to which the second immunoglobulin partner may be operatively linked by conventional means.

20 Fusion or linkage between the second immunoglobulin partners, e.g., antibody sequences, and the effector agent may be by any suitable means, e.g., by conventional covalent or ionic bonds, protein fusions, or hetero-bifunctional cross-linkers, e.g.,
25 carbodiimide, glutaraldehyde and the like. Such techniques are known in the art and are described in conventional chemistry and biochemistry texts.

30 Additionally, conventional linker sequences which simply provide for a desired amount of space between the second immunoglobulin partner and the effector agent may also be constructed into the altered immunoglobulin coding region. The design of such linkers is well known to those of skill in the art.

35 In addition, signal sequences for the molecules of the invention may be modified by techniques known to those skilled in the art to enhance expression.

A preferred altered antibody contains a variable heavy and/or light chain peptide or protein sequence having the antigen specificity of mAb BC2, e.g., the V_H and V_L chains. Still another desirable altered antibody of this invention is characterized by the amino acid sequence containing at least one, and preferably all of the CDRs of the variable region of the heavy and/or light chains of the murine antibody molecule BC2 with the remaining sequences being derived from a human source, or a functional fragment or analog thereof.

In a further embodiment, the altered antibody of the invention may have attached to it an additional agent. For example, recombinant DNA technology may be used to produce an altered antibody of the invention in which the Fc fragment or CH₂ CH₃ domain of a complete antibody molecule has been replaced by an enzyme or other detectable molecule (i.e., a polypeptide effector or reporter molecule).

The second immunoglobulin partner may also be operatively linked to a non-immunoglobulin peptide, protein or fragment thereof heterologous to the CDR-containing sequence having antigen specificity to a coagulation factor, preferably to Factor IX/IXa, X/Xa, XI/XIa, VIII/VIIIa, V/Va, VII/VIIa or thrombin/prothrombin. The resulting protein may exhibit both antigen specificity and characteristics of the non-immunoglobulin upon expression. That fusion partner characteristic may be, e.g., a functional characteristic such as another binding or receptor domain or a therapeutic characteristic if the fusion partner is itself a therapeutic protein or additional antigenic characteristics.

Another desirable protein of this invention may comprise a complete antibody molecule, having full length heavy and light chains or any discrete fragment thereof, such as the Fab or F(ab')₂ fragments, a heavy

chain dimer or any minimal recombinant fragments thereof such as an F_v or a single-chain antibody (SCA) or any other molecule with the same specificity as the selected donor mAb, e.g., mAb BC1, BC2, 9E4(2)F4, 11G4(1)B9, HFXLC or HFXI. Such protein may be used in the form of an altered antibody or may be used in its unfused form.

Whenever the second immunoglobulin partner is derived from an antibody different from the donor antibody, e.g., any isotype or class of immunoglobulin framework or constant regions, an engineered antibody results. Engineered antibodies can comprise immunoglobulin (Ig) constant regions and variable framework regions from one source, e.g., the acceptor antibody, and one or more (preferably all) CDRs from the donor antibody, e.g., the anti-Factor IX/IXa, X/Xa, XI/XIa, VIII/VIIIa, V/Va, VII/VIIa or thrombin/prothrombin antibodies described herein. In addition, alterations, e.g., deletions, substitutions, or additions, of the acceptor mAb light and/or heavy variable domain framework region at the nucleic acid or amino acid levels, or the donor CDR regions may be made in order to retain donor antibody antigen binding specificity.

Such engineered antibodies are designed to employ one (or both) of the variable heavy and/or light chains of the coagulation factor mAb (optionally modified as described) or one or more of the heavy or light chain CDRs. The engineered antibodies of the invention exhibit self-limiting neutralizing activity.

Such engineered antibodies may include a humanized antibody containing the framework regions of a selected human immunoglobulin or subtype or a chimeric antibody containing the human heavy and light chain constant regions fused to the coagulation factor antibody functional fragments. A suitable human (or other animal) acceptor antibody may be one selected from a

conventional database, e.g., the KABAT® database, Los Alamos database, and Swiss Protein database, by homology to the nucleotide and amino acid sequences of the donor antibody. A human antibody characterized by a homology
5 to the framework regions of the donor antibody (on an amino acid basis) may be suitable to provide a heavy chain variable framework region for insertion of the donor CDRs. A suitable acceptor antibody capable of donating light chain variable framework regions may be
10 selected in a similar manner. It should be noted that the acceptor antibody heavy and light chains are not required to originate from the same acceptor antibody.

Preferably, the heterologous framework and constant regions are selected from human immunoglobulin classes
15 and isotypes, such as IgG (subtypes 1 through 4), IgM, IgA, and IgE. However, the acceptor antibody need not comprise only human immunoglobulin protein sequences. For instance, a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain
20 is fused to a DNA sequence encoding a non-immunoglobulin amino acid sequence such as a polypeptide effector or reporter molecule.

A particularly preferred humanized antibody contains CDRs of BC2 inserted onto the framework regions
25 of a selected human antibody sequence. For neutralizing humanized antibodies, one, two or preferably three CDRs from the Factor IX antibody heavy chain and/or light chain variable regions are inserted into the framework regions of the selected human antibody sequence,
30 replacing the native CDRs of the latter antibody.

Preferably, in a humanized antibody, the variable domains in both human heavy and light chains have been engineered by one or more CDR replacements. It is possible to use all six CDRs, or various combinations of
35 less than the six CDRs. Preferably all six CDRs are replaced. It is possible to replace the CDRs only in

the human heavy chain, using as light chain the unmodified light chain from the human acceptor antibody. Still alternatively, a compatible light chain may be selected from another human antibody by recourse to the conventional antibody databases. The remainder of the engineered antibody may be derived from any suitable acceptor human immunoglobulin.

The engineered humanized antibody thus preferably has the structure of a natural human antibody or a fragment thereof, and possesses the combination of properties required for effective therapeutic use, e.g., treatment of thrombotic and embolic diseases in man.

Most preferably, the humanized antibodies have a heavy chain amino acid sequence as set forth in SEQ ID NO: 31, 52, or 89. Also most preferred are humanized antibodies having a light chain amino acid sequence as set forth in SEQ ID NO: 44, 57, 62, 74, 78 or 99. Particularly preferred is the humanized antibody SB 249413 where the heavy chain has the amino acid sequence as set forth in SEQ ID NO: 31 and the light chain has the amino acid sequence as set forth in SEQ ID NO: 44. Also particularly preferred is the humanized antibody SB 249415 where the heavy chain has the amino acid sequence as set forth in SEQ ID NO: 52 and the light chain has the amino acid sequence as set forth in SEQ ID NO: 57. Also particularly preferred is the humanized antibody SB 249416 where the heavy chain has the amino acid sequence as set forth in SEQ ID NO: 52 and the light chain has the amino acid sequence as set forth in SEQ ID NO: 62. Also particularly preferred is the humanized antibody SB 249417 where the heavy chain has the amino acid sequence as set forth in SEQ ID NO: 52 and the light chain has the amino acid sequence as set forth in SEQ ID NO: 74. Also particularly preferred is the humanized antibody SB 257731 where the heavy chain has the amino acid sequence as set forth in SEQ ID NO: 52 and the light chain has

the amino acid sequence as set forth in SEQ ID NO: 78. Also particularly preferred is the humanized antibody SB 257732 where the heavy chain has the amino acid sequence as set forth in SEQ ID NO: 89 and the light chain has
5 the amino acid sequence as set forth in SEQ ID NO: 99.

It will be understood by those skilled in the art that an engineered antibody may be further modified by changes in variable domain amino acids without necessarily affecting the specificity and high affinity
10 of the donor antibody (*i.e.*, an analog). It is anticipated that heavy and light chain amino acids may be substituted by other amino acids either in the variable domain frameworks or CDRs or both. These substitutions could be supplied by the donor antibody or
15 consensus sequences from a particular subgroup.

In addition, the constant region may be altered to enhance or decrease selective properties of the molecules of this invention. For example, dimerization, binding to Fc receptors, or the ability to bind and
20 activate complement (see, *e.g.*, Angal *et al.*, *Mol Immunol*, 30, 105-108 (1993), Xu *et al.*, *J Biol Chem*, 269, 3469-3474 (1994), Winter *et al.*, EP 307434-B).

An altered antibody which is a chimeric antibody differs from the humanized antibodies described above by
25 providing the entire non-human donor antibody heavy chain and light chain variable regions, including framework regions, in association with human immunoglobulin constant regions for both chains. It is anticipated that chimeric antibodies which retain
30 additional non-human sequence relative to humanized antibodies of this invention may elicit a significant immune response in humans.

Such antibodies are useful in the prevention and treatment of thrombotic and embolic disorders, as
35 discussed below.

Preferably, the variable light and/or heavy chain sequences and the CDRs of mAb BC2 or other suitable donor mAbs, e.g., BC1, 9E4(2)F4, 11G4(1)B9, HFXLC, HFXI, and their encoding nucleic acid sequences, are utilized in the construction of altered antibodies, preferably humanized antibodies, of this invention, by the following process. The same or similar techniques may also be employed to generate other embodiments of this invention.

10 A hybridoma producing a selected donor mAb, e.g., the murine antibody BC2, is conventionally cloned and the DNA of its heavy and light chain variable regions obtained by techniques known to one of skill in the art, e.g., the techniques described in Sambrook et al.,
15 "Molecular Cloning: A Laboratory Manual", 2nd edition, Cold Spring Harbor Laboratory (1989). The variable heavy and light regions of BC2 containing at least the CDR-encoding regions and those portions of the acceptor mAb light and/or heavy variable domain framework regions
20 required in order to retain donor mAb binding specificity, as well as the remaining immunoglobulin-derived parts of the antibody chain derived from a human immunoglobulin, are obtained using polynucleotide primers and reverse transcriptase. The CDR-encoding
25 regions are identified using a known database and by comparison to other antibodies.

A mouse/human chimeric antibody may then be prepared and assayed for binding ability. Such a chimeric antibody contains the entire non-human donor
30 antibody V_H and V_L regions, in association with human Ig constant regions for both chains.

Homologous framework regions of a heavy chain variable region from a human antibody are identified using computerized databases, e.g., KABAT®, and a human
35 antibody having homology to BC2 is selected as the acceptor antibody. The sequences of synthetic heavy

chain variable regions containing the BC2 CDR-encoding regions within the human antibody frameworks are designed with optional nucleotide replacements in the framework regions to incorporate restriction sites.

5 This designed sequence is then synthesized using long synthetic oligomers. Alternatively, the designed sequence can be synthesized by overlapping oligonucleotides, amplified by polymerase chain reaction (PCR), and corrected for errors. A suitable light chain
10 variable framework region can be designed in a similar manner.

A humanized antibody may be derived from the chimeric antibody, or preferably, made synthetically by inserting the donor mAb CDR-encoding regions from the
15 heavy and light chains appropriately within the selected heavy and light chain framework. Alternatively, a humanized antibody of the invention may be prepared using standard mutagenesis techniques. Thus, the resulting humanized antibody contains human framework
20 regions and donor mAb CDR-encoding regions. There may be subsequent manipulation of framework residues. The resulting humanized antibody can be expressed in recombinant host cells, e.g., COS, CHO or myeloma cells. Other humanized antibodies may be prepared using this
25 technique on other suitable Factor IX-specific or other coagulation factor-specific, self-limiting, neutralizing, high affinity, non-human antibodies.

A conventional expression vector or recombinant plasmid is produced by placing these coding sequences
30 for the altered antibody in operative association with conventional regulatory control sequences capable of controlling the replication and expression in, and/or secretion from, a host cell. Regulatory sequences include promoter sequences, e.g., CMV promoter, and
35 signal sequences, which can be derived from other known antibodies. Similarly, a second expression vector can

be produced having a DNA sequence which encodes a complementary antibody light or heavy chain. Preferably, this second expression vector is identical to the first except with respect to the coding sequences and selectable markers, in order to ensure, as much as possible, that each polypeptide chain is functionally expressed. Alternatively, the heavy and light chain coding sequences for the altered antibody may reside on a single vector.

10 A selected host cell is co-transfected by conventional techniques with both the first and second vectors (or simply transfected by a single vector) to create the transfected host cell of the invention comprising both the recombinant or synthetic light and heavy chains. The transfected cell is then cultured by conventional techniques to produce the engineered antibody of the invention. The humanized antibody which includes the association of both the recombinant heavy chain and/or light chain is screened from culture by an appropriate assay such as ELISA or RIA. Similar conventional techniques may be employed to construct other altered antibodies and molecules of this invention.

Suitable vectors for the cloning and subcloning steps employed in the methods and construction of the compositions of this invention may be selected by one of skill in the art. For example, the pUC series of cloning vectors, such as pUC19, which is commercially available from supply houses, such as Amersham or Pharmacia, may be used. Additionally, any vector which is capable of replicating readily, has an abundance of cloning sites and selectable genes (e.g., antibiotic resistance) and is easily manipulated may be used for cloning. Thus, the selection of the cloning vector is not a limiting factor in this invention.

Similarly, the vectors employed for expression of the engineered antibodies according to this invention may be selected by one of skill in the art from any conventional vector. The vectors also contain selected regulatory sequences (such as CMV promoters) which direct the replication and expression of heterologous DNA sequences in selected host cells. These vectors contain the above-described DNA sequences which code for the engineered antibody or altered immunoglobulin coding region. In addition, the vectors may incorporate the selected immunoglobulin sequences modified by the insertion of desirable restriction sites for ready manipulation.

The expression vectors may also be characterized by genes suitable for amplifying expression of the heterologous DNA sequences, e.g., the mammalian dihydrofolate reductase gene (DHFR). Other preferable vector sequences include a poly A signal sequence, such as from bovine growth hormone (BGH) and the betaglobin promoter sequence (betaglopro). The expression vectors useful herein may be synthesized by techniques well known to those skilled in this art.

The components of such vectors, e.g., replicons, selection genes, enhancers, promoters, signal sequences and the like, may be obtained from commercial or natural sources or synthesized by known procedures for use in directing the expression and/or secretion of the product of the recombinant DNA in a selected host. Other appropriate expression vectors of which numerous types are known in the art for mammalian, bacterial, insect, yeast and fungal expression may also be selected for this purpose.

The present invention also encompasses a cell line transfected with a recombinant plasmid containing the coding sequences of the engineered antibodies or altered immunoglobulin molecules thereof. Host cells useful for

the cloning and other manipulations of these cloning vectors are also conventional. However, most desirably, cells from various strains of *E. coli* are used for replication of the cloning vectors and other steps in the construction of altered antibodies of this invention.

Suitable host cells or cell lines for the expression of the engineered antibody or altered antibody of the invention are preferably mammalian cells such as CHO, COS, a fibroblast cell (e.g., 3T3) and myeloid cells, and more preferably a CHO or a myeloid cell. Human cells may be used, thus enabling the molecule to be modified with human glycosylation patterns. Alternatively, other eukaryotic cell lines may be employed. The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. See, e.g., Sambrook *et al.*, *supra*.

Bacterial cells may prove useful as host cells suitable for the expression of the recombinant Fabs of the present invention (see, e.g., Plückthun, A., *Immunol Rev*, 130, 151-188 (1992)). However, due to the tendency of proteins expressed in bacterial cells to be in an unfolded or improperly folded form or in a non-glycosylated form, any recombinant Fab produced in a bacterial cell would have to be screened for retention of antigen binding ability. If the molecule expressed by the bacterial cell was produced in a properly folded form, that bacterial cell would be a desirable host. For example, various strains of *E. coli* used for expression are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis*, *Streptomyces*, other bacilli and the like may also be employed.

Where desired, strains of yeast cells known to those skilled in the art are also available as host cells, as well as insect cells, e.g. *Drosophila* and *Lepidoptera* and viral expression systems. See, e.g. Miller *et al.*, *Genetic Engineering*, 8, 277-298, Plenum Press (1986) and references cited therein.

The general methods by which the vectors of the invention may be constructed, the transfection methods required to produce the host cells of the invention, and culture methods necessary to produce the altered antibody of the invention from such host cells are all conventional techniques. Likewise, once produced, the altered antibodies of the invention may be purified from the cell culture contents according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like. Such techniques are within the skill of the art and do not limit this invention.

Yet another method of expression of the humanized antibodies may utilize expression in a transgenic animal, such as described in U. S. Patent No. 4,873,316. This relates to an expression system using the animal's casein promoter which when transgenically incorporated into a mammal permits the female to produce the desired recombinant protein in its milk.

Once expressed by the desired method, the engineered antibody is then examined for *in vitro* activity by use of an appropriate assay. Presently, conventional ELISA assay formats are employed to assess qualitative and quantitative binding of the engineered antibody to Factor IX or to other appropriate coagulation factors. Additionally, other *in vitro* assays may also be used to verify neutralizing efficacy prior to subsequent human clinical studies performed to

evaluate the persistence of the engineered antibody in the body despite the usual clearance mechanisms.

Following the procedures described for humanized antibodies prepared from BC2, one of skill in the art
5 may also construct humanized antibodies from other donor antibodies, variable region sequences and CDR peptides described herein. Engineered antibodies can be produced with variable region frameworks potentially recognized as "self" by recipients of the engineered antibody.
10 Minor modifications to the variable region frameworks can be implemented to effect large increases in antigen binding without appreciable increased immunogenicity for the recipient. Such engineered antibodies may effectively treat a human for coagulation factor-
15 mediated conditions. Such antibodies may also be useful in the diagnosis of such conditions.

This invention also relates to a method for inhibiting thrombosis in an animal, particularly a human, which comprises administering an effective dose
20 of an anti-coagulation factor monoclonal antibody having self-limiting neutralizing activity in combination with a plasminogen activator. Combination therapy enhances thrombolysis at sub-optimal concentrations of plasminogen activator decreasing the time to restoration
25 of blood flow and increasing the frequency and total duration of vessel reperfusion. In contrast to heparin, combination therapy does not significantly perturb normal hemostatic functions and spares fibrinogen, plasminogen and alpha-2-antiplasmin levels.
30 Accordingly, this invention also relates to a method of reducing a required dose of a thrombolytic agent in treatment of thrombosis in an animal comprising administering an anticoagulant specifically targeting a component of the intrinsic coagulation pathway in
35 combination with the thrombolytic agent.

Preferably, the coagulation factor is from the intrinsic or common coagulation pathway. Most preferably, the anti-coagulation factor monoclonal antibody is an anti-Factor IX, anti-Factor IXa, anti-Factor X, anti-Factor Xa, anti-Factor XI, anti-Factor XIa, anti-Factor VIII, anti-Factor VIIIA, anti-Factor V, anti-Factor Va, anti-Factor VII, anti-Factor VIIa, anti-thrombin or anti-prothrombin. The mAb can include one or more of the engineered antibodies or altered antibodies described herein or fragments thereof. Preferably, the plasminogen activator is tPA, streptokinase, urokinase or tPA variants as described in, e.g., Tachias and Madison, *J Biol Chem*, 272, 14580-14585 (1997); Fujise et al., *Circulation*, 95, 715-722 (1997); Coombs et al., *J Biol Chem*, 273, 4323-4328 (1998); Van de Werf et al., *Am Heart J*, 137, 786-791 (1999) . Particularly preferred is tPA.

Alternatively, acetylsalicylic acid can be administered in combination with the anti-coagulation factor monoclonal antibody. In some cases, combination therapy lowers the therapeutically effective dose of anti-coagulation factor monoclonal antibody.

The therapeutic response induced by the use of the molecules of this invention is produced by the binding to the respective coagulation factor and the subsequent self-limiting inhibition of the coagulation cascade. Thus, the molecules of the present invention, when in preparations and formulations appropriate for therapeutic use, are highly desirable for persons susceptible to or experiencing abnormal clotting activity associated with, but not limited to, myocardial infarction, unstable angina, atrial fibrillation, stroke, renal damage, pulmonary embolism, deep vein thrombosis and artificial organ and prosthetic implants. A particularly preferred use is in myocardial infarction.

The altered antibodies, antibodies and fragments thereof of this invention may also be used in conjunction with other antibodies, particularly human mAbs reactive with other markers (epitopes) responsible for the condition against which the engineered antibody of the invention is directed.

The therapeutic agents of this invention are believed to be desirable for treatment of abnormal clotting conditions from about 1 day to about 3 weeks, or as needed. This represents a considerable advance over the currently used anticoagulants heparin and warfarin. The dose and duration of treatment relates to the relative duration of the molecules of the present invention in the human circulation, and can be adjusted by one of skill in the art depending upon the condition being treated and the general health of the patient.

The mode of administration of the therapeutic agents of the invention may be any suitable route which delivers the agent to the host. The altered antibodies, antibodies, engineered antibodies, and fragments thereof, plasminogen activator and pharmaceutical compositions of the invention are particularly useful for parenteral administration, *i.e.*, subcutaneously, intramuscularly, intravenously or intranasally.

Therapeutic agents of the invention may be prepared as pharmaceutical compositions containing an effective amount of the engineered (e.g., humanized) antibody of the invention and plasminogen activator as active ingredients in a pharmaceutically acceptable carrier. Alternatively, the pharmaceutical compositions of the invention could also contain acetylsalicylic acid. In the prophylactic agent of the invention, an aqueous suspension or solution containing the engineered antibody, preferably buffered at physiological pH, in a form ready for injection is preferred. The compositions for parenteral administration will commonly comprise a

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present in unit dose forms. The appropriate therapeutically effective dose can be determined readily by those of skill in the art. To effectively treat a thrombotic or embolic disorder in a human or other animal, one dose of approximately 0.1 mg to approximately 20 mg per kg body weight of a protein or an antibody of this invention should be administered parenterally, preferably *i.v.* or *i.m.* Such dose may, if necessary, be repeated at appropriate time intervals selected as appropriate by a physician during the thrombotic response.

The antibodies, altered antibodies or fragments thereof described herein can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immunoglobulins and art-known lyophilization and reconstitution techniques can be employed.

The present invention will now be described with reference to the following specific, non-limiting examples.

Example 1Preparation and Screening of Anti-Factor IX Monoclonal
Antibodies

Female Balb/C mice were injected with human factor
5 IX purified as described in Jenny, R. *et al.*, *Prep*
Biochem, 16, 227-245 (1986). Typically, each mouse
received an initial injection of 100 ug protein
dissolved in 0.15 mL phosphate-buffered saline (PBS) and
mixed with 0.15 mL complete Freund's adjuvant. Booster
10 immunizations of 50 ug protein in 0.15 mL PBS with 0.15
mL incomplete Freund's adjuvant were given approximately
biweekly over a 2-3 month period. After the final
boost, the mouse received 50 ug of Factor IX in PBS
three days before spleen/myeloma cell fusions. Spleen
15 cells were isolated from an immunized mouse and fused
with NS-1 myeloma cells (Kohler, G. *et al.*, *Eur J*
Immunol, 6, 292-295 (1976)) using polyethylene glycol as
described by Oi, V.T. *et al.* in "Selected Methods in
Cellular Immunology," Mishell, B.B. and Shigii, S.M.,
20 eds., Freeman Press, San Francisco. Following the
fusion, the cells were resuspended in RPMI 1640 media
containing 10% fetal calf sera and aliquots were placed
in each well of four 24-well plates containing 0.5 mL of
peritoneal lavage cell-conditioned media. On the
25 following day, each well received 1.0 mL of 2×10^{-4} M
hypoxanthine, 8×10^{-7} M aminopterin and 3.2×10^{-5} M
thymidine in RPMI 1640 media containing 10% fetal calf
sera. The cells were fed every 3-4 days by removing
half of the media and replacing it with fresh media
30 containing 1×10^{-4} M hypoxanthine and 1.6×10^{-5} M
thymidine.

Approximately two weeks later, 1.0 mL of hybridoma
medium was removed from each well and tested for anti-
Factor IX antibodies using an ELISA assay as described
35 by Jenny, R.J. *et al.* in *Meth Enzymol*, 222, 400-416
(1993). Briefly, factor IX was immobilized onto

plastic wells of 96-well microtiter plates. Hybridoma supernatants or dilutions of purified antibody were then incubated in the wells. The wells were washed and the presence of antibody-antigen complexes detected with a
5 goat anti-murine immunoglobulin second antibody conjugated to horseradish peroxidase and the chromogenic substrate o-dianisidine.

Wells containing anti-Factor IX antibodies were subcloned by limiting dilution and grown in 96-well
10 plates. Supernatant from the cloned hybridoma cell cultures were screened for antibody to Factor IX by the ELISA assay described above and cells from positive hybridomas were expanded, frozen, stored in liquid nitrogen and then grown as ascitic tumors in mice.

Example 2

Self-Limiting Effect of Anti-Coagulation Factor

Antibodies in Coagulation

The effect of increasing concentrations of anti-
20 coagulation factor antibodies on activated partial thromboplastin time (aPTT) of human plasma was determined in a fibrometer (Becton-Dickinson Microbiology Systems, Cockeysville, Maryland) using Baxter reference procedure LIB0293-J, 3/93 revision
25 (Baxter Scientific, Edison, New Jersey).

Prior to the start of the experiment, 2 to 3 mL of 0.02 M CaCl_2 in a 5 mL tube were placed into the heating chamber of the fibrometer. Human plasma samples were either freshly drawn and kept on ice or reconstituted
30 per the manufacturer's recommendation from Hemostasis Reference Plasma (American Diagnostics, Greenwich, Connecticut).

Unfractionated heparin from porcine intestinal mucosa (Sigma Chemical, St. Louis, Missouri), low
35 molecular weight heparin from porcine intestinal mucosa (Lovenox®, enoxaparin sodium, Rhone-Poulenc Rorer

Pharmaceuticals, Collegeville, Pennsylvania) or mAb anticoagulants were prepared as approximately 50 uM stock solutions and serially diluted directly into the test plasma. A blank containing plasma without
5 anticoagulant was included as a reference.

Two fibroTube® fibrometer cups were filled with 100 ul test plasma or 100 ul test plasma with anticoagulant and 125 ul of actin activated cephaloplastin reagent (Actin reagent, from rabbit brain cephalin in ellagic
10 acid, available from Baxter Scientific), respectively and placed in the fibrometer wells at 37°C.

After one minute, 100 ul of actin reagent was transferred to a plasma-containing cup and the contents mixed several times with a pipette. After a 3 minute
15 incubation, 100 ul of CaCl_2 , prewarmed at 37°C, was added to the plasma-actin reagent mixture using a Automatic Pipette/Timer-trigger (Becton-Dickinson). The clotting times were noted and the results in Fig. 1 are presented as clotting times as a function of final concentrations
20 of anticoagulant in the total assay volume of 300 ul. The nominal concentration of Factor IX in the assay is 30-40 nM.

The results shown in Fig. 1 demonstrate the effect of increasing concentrations of the murine anti-Factor
25 IX mAbs BC1 and BC2 on aPTT clotting times. Both mAbs inhibit clotting by prolonging the aPTT and both mAbs reach a final saturating effect on the aPTT. The IC_{50} values are similar at ~35 nM and ~50 nM for BC1 and BC2, respectively, but the difference in the maximum response
30 to the two antibodies is marked. Saturating concentrations of BC1 increases the aPTT by about 50% to ~40 sec. BC2, on the other hand, increases the aPTT by 3.5-fold to about 90 sec. The therapeutic target zone used in anticoagulant therapy with heparin is
35 highlighted. The results indicate that the two mAbs bracket the heparin therapeutic aPTT range.

The properties of mAbs BC1 and BC2 are summarized in Table I. Each of the BC mAbs recognizes both the zymogen, Factor IX, as well as the active protease, Factor IXa, but only BC2 is capable of blocking both zymogen activation as well as protease activity. BC1 and BC2 were found to cross-react with Cynomologous monkey Factor IX. Additionally, BC2 also cross-reacted with rat Factor IX.

Table I. Summary of *in vitro* Properties of Anti-Factor IX mAbs

	BC1	BC2
Binds Factor IX	yes	yes
Binds Factor IXa	yes	yes
Inhibits IX to IXa conversion	no	yes
Inhibits IXa activity in Xase complex	yes	yes
Cofactor requirement	none	divalent metals
		$\text{Ca}^{2+} > \text{Mn}^{2+}$
$\frac{\text{aPTTmax}}{\text{aPTTnormal}} \times 100\%$	150	350
IC_{50} , nM	~35	~50
Species cross-reactivity	monkey	rat, monkey
Isotype	IgG1	IgG2a

The results shown in Fig. 2 demonstrate the effect of increasing concentrations of the anti-Factor IX mAbs 9E4(2)F4 and 11G4(1)B9 on aPTT clotting times. The plasma for the assay was diluted to one-half the normal concentration, giving an initial aPTT of 45 seconds. Both mAbs inhibit clotting by prolonging the aPTT and both mAbs reach a final saturating effect on the aPTT. Saturating concentrations of 9E4(2)F4 and 11G4(1)B9 increases the aPTT to ~90 to 100 seconds for 9E4(2)F4 and to ~80 seconds for 11G4(1)B9. The results indicate that the two mAbs are at the upper end of the heparin therapeutic aPTT range.

The results shown in Fig. 3 demonstrate the effect of increasing concentrations of the anti-Factor X mAbs HFXLC (vs. light chain epitope), HFXHC (vs. heavy chain epitope) and the anti-Factor XI mAb HFXI on aPTT clotting times. These mAbs were obtained from Enzyme Research Laboratories (South Bend, IN). The mAbs HFXLC and HFXI inhibit clotting by prolonging the aPTT and both mAbs reach a final saturating effect on the aPTT. The IC_{50} value for HFXLC is ~40 nM; saturating concentrations increase the aPTT to ~60 seconds. The IC_{50} value for HFXI is ~20 nM; saturating concentrations increase the aPTT to ~100 seconds. The results indicate that HFXLC is within the heparin therapeutic aPTT range while HFXI falls at the upper end of the heparin therapeutic range. The mAb HFXHC had no effect on aPTT clotting times.

Self-limiting prolongation of the aPTT was also observed with antibodies to Factor VIII, the cofactor to Factor IXa. For example, the anti-human Factor VIII antibody, SAF8C-IG, purchased from Affinity Biologicals, Inc., increased the aPTT to a maximum of about 65 sec. Half-maximal prolongation of the aPTT was achieved with about 100 nM antibody.

Example 3

Efficacy of murine Factor IX mAbs in Rat Thrombus Model

In order to evaluate the efficacy of anti-Factor IX antibodies in prevention of arterial thrombosis, the rat carotid artery thrombosis model as reported by Schumacher *et al.* in *J Cardio Pharm*, 22, 526-533 (1993) was adapted. This model consists of segmental injury to the carotid endothelium by oxygen radicals generated by FeCl₃ solution applied on the surface of the carotid artery.

In brief, rats were anesthetized with pentobarbitone sodium, the jugular vein cannulated for intravenous injections and the left femoral artery cannulated for blood pressure and heart rate monitoring. The carotid artery was isolated by aseptic technique via a surgical incision in the neck and equipped with a magnetic flow probe for blood flow measurement. After a period of stabilization, baseline parameters were established for the following variables: carotid blood flow, arterial pressure, heart rate, activated partial thromboplastin time (aPTT) and prothrombin time (PT). Thereafter, a premeasured Whatman filter paper soaked in 50% FeCl₃ solution was placed on the carotid artery for 15 minutes for complete injury of the underlying endothelial cells. After removal of the FeCl₃ soaked paper, the experiment was followed to completion over 60 minutes. At the end of the experiment, the carotid thrombus was extracted from the carotid artery and weighed.

All agents were administered 15 minutes prior to the onset of carotid injury. The following treatments were examined and compared to the Factor IX mAb BC2.

1. Heparin: 15, 30, 60 or 120 U/kg bolus, followed by infusion of 0.5, 1, 2 or 4 U/kg/min, respectively over 60 minutes

2. Acetylsalicylic acid (ASA, aspirin): 5 mg/kg bolus

3. Anti-Factor IX mAb BC2: 1, 3 or 6 mg/kg bolus, followed by infusion 0.3, 1, or 2 ug/kg/min, respectively over 60 minutes

4. Heparin: 30U/kg bolus + 1U/kg/min + ASA at 5 mg/kg

5. Anti-Factor IX mAb BC2: 1 mg/kg + 0.3 ug/kg/min + ASA at 5 mg/kg

Figs. 4 and 5 demonstrate the comparative pharmacology of the anti-coagulant/thrombotic regimens by showing the effect of heparin, ASA and Factor IX mAb BC2 on aPTT (Fig. 4) and PT (Fig. 5).

The key index for bleeding diathesis, aPTT, was used as the primary criterion for evaluation of efficacy versus bleeding liabilities of the anti-coagulant/thrombotic agents used in the study. The results in Fig. 4 demonstrate the dose-dependent prolongation of aPTT by heparin with maximal prolongation of the clotting time, beyond the test limit, at the two higher doses. ASA alone did not significantly increase aPTT but in combination with heparin, a marked synergistic effect was observed. The Factor IX mAbs had a modest effect on aPTT and even at the highest dose, the increase in clotting time did not exceed the 3-fold limit of standard anti-coagulant practiced clinically. Most notably, the low dose of Factor IX mAb BC2 in combination with ASA did not change the aPTT.

In Fig. 5, the data indicate that PT was also significantly prolonged by heparin, at the two higher doses, and by the ASA + heparin combination, but not by any of the Factor IX mAb doses alone or in combination with ASA.

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The effect of heparin, ASA and Factor IX mAb on
carotid artery occlusion is shown in Fig. 6. The
results indicate that the carotid arteries of all of the
vehicle-treated animals occlude in response to the
5 injury. Heparin dose dependently inhibited the
occlusion of the carotid artery. At the highest dose,
heparin completely prevented the occlusion of the
carotid artery; at this dose however, no coagulation
could be initiated. ASA alone had only a minor effect
10 on carotid occlusion. ASA in combination with heparin
also failed to completely prevent carotid occlusion.
Factor IX mAb completely blocked carotid occlusion at
the two higher doses, which have not prolonged
coagulation beyond the clinically desired target. The
15 lower dose of Factor IX mAb, that largely failed to
secure patency alone, demonstrated complete inhibition
of carotid occlusion when administered in combination
with ASA.

The effect of heparin, ASA and Factor IX mAb on
20 thrombus weight is shown in Fig. 7. Heparin dose-
dependently reduced thrombus mass in the carotid
artery. However, some residual thrombus was still found
in the carotid artery in spite of complete blockade of
coagulation. ASA alone or in combination with heparin
25 (30 U/kg regimen) had only a partial effect on thrombus
weight. Factor IX mAb dose-dependently reduced thrombus
mass and the high dose virtually prevented completely
thrombus formation. Moreover, the combination of the
low dose anti-Factor IX mAb and ASA, a regimen that
30 completely prevented carotid occlusion without adversely
affecting the coagulation indices, completely prevented
thrombus formation.

The studies conducted in the rat carotid thrombosis
model clearly demonstrate the efficacy of Factor IX mAb
35 in prevention of thrombosis in a highly thrombogenic
arterial injury model. Most notably, the efficacy of

the Factor IX mAb was demonstrated within the desired therapeutic anticoagulant target defined by the aPTT. Furthermore, heparin, the current standard anticoagulant, reached efficacy comparable to Factor IX mAb only at doses that severely compromised coagulation to the extent of producing non-coagulable blood. Interestingly, the observed potentiation and synergy acquired by ASA joint treatment with heparin was also demonstrated when ASA was given with anti-Factor IX mAb. However, unlike the combination of heparin and ASA which resulted in potentiation of both the anti-thrombotic and anti-coagulant effects, the combination of Factor IX mAb and ASA resulted in potentiation of the anti-thrombotic efficacy with no consistent effect on ex vivo blood coagulation parameters. Taken together, the data show a superior antithrombotic capacity of Factor IX mAb compared to heparin, ASA or a combination of heparin and ASA.

Example 4

Scanning Electron Microscopy of Rat Thrombosis Model

Segments of rat carotid artery were collected from sham, ferric chloride only and ferric chloride + 6 mg/kg Factor IX antibody, 3/group, 15 minutes after application of ferric chloride. The arteries were fixed by perfusion with formaldehyde and ligated above and below the lesioned area. Fixed arteries were dehydrated, incubated in hexamethyldisilazane and dried in a desiccator. Dried arteries were opened lengthwise, placed on Scanning Electron Microscopy (SEM) stubs and sputter coated with gold.

SEM of sham arteries revealed an essentially normal endothelium with rare scattered platelets. There were a few breaks in the endothelium, probably as a result of mechanical damage during surgery and the underlying basement membrane was covered by a carpet of platelets.

No evidence of thrombus formation was observed in the sham rats.

SEM of the arteries treated with ferric chloride revealed large mural thrombi which occupied a large portion of the lumen of the vessel. The thrombi were composed of aggregated platelets, red blood cells and amorphous and fibrillar proteinaceous material. The proteinaceous material is consistent with fibrin. The endothelium of the arteries was mostly obscured by the large thrombi. Where visible, the endothelium overlying the region treated with ferric chloride was covered by numerous adherent platelets and amorphous proteinaceous material.

SEM of the arteries treated with ferric chloride from rats also treated with Factor IX antibody, revealed the lumen of the vessels to be largely free of thrombus. The endothelium overlying the region treated with ferric chloride showed extensive damage and some areas were covered by adherent platelets and platelet aggregates but there was little or no proteinaceous material.

Example 5

Anti-Factor IX mAb BC2 Heavy and Light Chain cDNA

Sequence Analysis

Total RNA was purified by using TriReagent (Molecular Research Center, Inc., Cincinnati, OH) according to the manufacturer's protocol. RNA was precipitated with isopropanol and dissolved in 0.5% SDS and adjusted to 0.5M NaCl. Poly A⁺ RNA was isolated with Dynabeads Oligo (dT)₂₅ (DynaL A.S., Lake Success, NY) according to the manufacturer's protocol. Poly A⁺ RNA was eluted from the beads and resuspended in TE buffer. Twelve aliquots of 100 ng of RNA were reverse transcribed with a RT-PCR kit per the manufacturer's instructions (Boehringer Mannheim Cat. No. 1483-188) using a dT oligo for priming. For the heavy chain, PCR

amplifications of 6 RNA/DNA hybrids were carried out for 25 cycles using a murine IgG2a hinge primer (SEQ ID NO: 1) and a heavy chain signal sequence primer (SEQ ID NO: 2). Similarly, for the light chain, PCR amplifications of 6 RNA/DNA hybrids were carried out for 25 cycles using a murine kappa primer (SEQ ID NO: 3) and a degenerate light chain signal sequence primer (SEQ ID NO: 4). The PCR products from each of the 12 amplifications were ligated in a PCR2000 vector (TA cloning Kit, Invitrogen, Cat. No. K2000-01). Colonies of recombinant clones were randomly picked and minipreparations of plasmid DNA were prepared using an alkaline extraction procedure described by Birnboim and Doly in *Nucl. Acids Res.* 7, 1513 (1979). The isolated plasmid DNA was digested with *EcoRI* and analyzed on a 0.8% agarose gel. Double-stranded cDNA inserts of the appropriate size, i.e., ~700 bp for the heavy chain and ~700 bp for the light chain, were sequenced by a modification of the Sanger method. The sequence of all 12 of the heavy and light chains were compared to generate a consensus BC2 heavy chain variable region sequence (SEQ ID NO: 5) and consensus BC2 light chain variable region sequence (SEQ ID NO: 6).

Sequence analysis of the BC2 heavy chain variable region cDNA revealed a 363 nucleotide open reading frame encoding a 121 amino acid sequence (SEQ ID NO: 7). The heavy chain CDR1, 2 and 3 sequences are listed in SEQ ID NOs: 8, 9 and 10, respectively.

Sequence analysis of the BC2 light chain variable region cDNA revealed a 321 nucleotide open reading frame encoding a 107 amino acid sequence (SEQ ID NO: 11). The light chain CDR1, 2 and 3 sequences are listed in SEQ ID NOs: 12, 13 and 14, respectively.

Example 6**Humanized Antibodies**

Six humanized antibodies designated SB 249413, SB 249415, SB 249416, SB249417, SB 257731 and SB 257732 were designed to contain the murine CDRs described above in a human antibody framework.

SB 249413

SB 249413 contains the heavy chain F9HZHC 1-0 and the light chain F9HZLC 1-0. The synthetic variable region humanized heavy chain F9HZHC 1-0 was designed using the first three framework regions of the heavy chain obtained from immunoglobulin RF-TS3'CL (Capra, J.D. *et al.*, *J. Clin. Invest.* 86, 1320-1328 (1990) identified in the Kabat database as Kabpro:Hhc10w) and the BC2 heavy chain CDRs described previously. No framework amino acids substitutions which might influence CDR presentation were made. Four overlapping synthetic oligonucleotides were generated (SEQ ID NOs: 15, 16, 17 and 18) which, when annealed and extended, code for the amino acids representing the heavy chain variable region through and including CDR3 (SEQ ID NOs: 19 and 20). This synthetic gene was then amplified using PCR primers (SEQ ID NOs: 21 and 22) and ligated into the pCR2000 vector (TA cloning Kit, Invitrogen, Cat. No. K2000-01) and isolated from a *SpeI*, *KpnI* restriction digest. A second DNA fragment coding for the campath signal sequence including the first five amino acids of the variable region (SEQ ID NOs: 23 and 24) was made by PCR amplification of the appropriate region of a construct encoding a humanized anti-Respiratory Syncytial Virus heavy chain (SEQ ID NO: 25) with two primers (SEQ ID NOs: 26 and 27) and digesting with the restriction enzymes *EcoRI* and *SpeI*. The two fragments generated were ligated into an *EcoRI*, *KpnI* digested pFHZHC2-6pCD mammalian cell expression vector which contained the remainder of a human consensus

framework 4 and IgG1 constant region. The vector contained a single amino acid mutation of the pFHZHC2-3pCD vector described in published International Patent Application No. WO94/05690. The final residue of
5 framework 2 (residue 49) was mutated from Ser to Ala by digesting pFHZHC2-3pCD with *Xba*I and *Eco*R5 and inserting a linker generated from two synthetic oligonucleotides (SEQ ID NOs: 28 and 29). The sequence of the F9HZHC 1-0 insert is shown in SEQ ID NOs: 30 and 31.

10 The synthetic variable region humanized light chain F9HZLC 1-0 was designed using the framework regions of the human light chain obtained from immunoglobulin LS8'CL (Carmack *et al.*, *J. Exp. Med.* 169, 1631-1643 (1989) identified in the Kabat database as
15 Kabpro:Hkl318) and the BC2 light chain CDRs described previously. No framework amino acids substitutions which might influence CDR presentation were made. Two overlapping synthetic oligonucleotides were generated (SEQ ID NOs: 32 and 33) which, when annealed and
20 extended, code for amino acids representing the light chain variable region (SEQ ID NOs: 34 and 35). This synthetic gene was then amplified using PCR primers (SEQ ID NOs: 36 and 37) and ligated into the pCR2000 vector (TA cloning Kit, Invitrogen, Cat. No. K2000-01), and
25 isolated from a *Sca*I, *Sac*II restriction digest. A second DNA fragment coding for the campath signal sequence including the first two amino acids of the variable region (SEQ ID NOs: 38 and 39) was made by PCR amplification of the the appropriate region of a
30 construct encoding a humanized anti-Respiratory Syncytial Virus heavy chain (SEQ ID NO: 25) with the two primers (SEQ ID NOs: 26 and 40) and digesting with the restriction enzymes *Eco*RI and *Sca*I. The two fragments generated were ligated into an *Eco*R1, *Sac*II digested
35 pFHzLC1-2pCN mammalian cell expression vector which contained the remainder of a human framework 4 and kappa

constant region. The vector contained a single amino acid mutation of the pFHZLC1-1pCN vector described in published International Patent Application No.

WO94/05690. A framework 2 residue was mutated from Ser to Pro by digesting pFHZLC1-pCN with *Sma*I and *Kpn*I and inserting a linker generated from two synthetic oligonucleotides (SEQ ID NOs: 41 and 42). The sequence of the F9HZLC 1-0 insert is shown in SEQ ID NOs: 43 and 44.

10 **SB 249415**

SB 249415 contains the heavy chain F9HZHC 1-1 and the light chain F9HZLC 1-1. These heavy and light chain constructs are based on F9HZHC 1-0 and F9HZLC 1-0, respectively, however, they have framework amino acid substitutions which can influence CDR presentation.

F9HZHC 1-1 has three framework amino acid substitutions which might influence CDR presentation. Two overlapping synthetic oligonucleotides were generated (SEQ ID NOs: 45 and 46), which when annealed and extended, code for amino acids representing the altered portion of the heavy chain variable region altered (SEQ ID NOs: 47 and 48). This synthetic gene was then amplified using PCR primers (SEQ ID NOs: 49 and 50), ligated into the pCR2000 vector (TA cloning Kit, Invitrogen, Cat. No. K2000-01) and isolated from a *Eco*NI, *Kpn*I restriction digest. This fragment was ligated into *Eco*NI, *Kpn*I digested F9HZHC1-0 (SEQ ID NO: 30) vector. The sequence of the F9HZHC 1-1 insert is shown in SEQ ID NOs: 51 and 52.

F9HZLC 1-1 has four framework amino acids substitutions which can influence CDR presentation. Two synthetic oligonucleotides were generated (SEQ ID NOs: 53 and 54), which when annealed, have *Kpn*I and *Bam*HI cohesive ends, and code for amino acids representing the altered portion of the light chain variable region (SEQ ID NO: 55). F9HZLC 1-0 (SEQ ID NO: 43) was digested

with the restriction enzymes *KpnI* and *BamHI* and ligated to the synthetic DNA. The sequence of the F9HZLC 1-1 insert is shown in SEQ ID NOs: 56 and 57.

SB 249416

5 SB 249416 contains the heavy chain F9HZHC 1-1 (described above) (SEQ ID NO: 52) and the light chain F9HZLC 1-2. The light chain construct is based on F9HZLC 1-1, however, it has one additional framework amino acid substitution which can influence CDR
10 presentation.

Two synthetic oligonucleotides were generated (SEQ ID NOs: 58 and 59), which when annealed, have *BamHI* and *XbaI* cohesive ends and code for amino acids representing the altered portion of the light chain variable region
15 (SEQ ID NO: 60). F9HZLC 1-1 (SEQ ID NO: 56) vector was digested with the restriction enzymes *BamHI* and *XbaI* and ligated to the synthetic DNA. The sequence of the F9HZLC 1-2 insert is shown in SEQ ID NOs: 61 and 62.

SB 249417

20 SB 249417 contains the heavy chain F9HZHC 1-1 (described above) (SEQ ID NO: 52) and the light chain F9HZLC 2-0. A F9HZLC 2-0 synthetic variable region humanized light chain was designed using the framework regions of the human light chain obtained from
25 immunoglobulin REI (Palm and Hilschmann, *Z. Physiol. Chem.* 354, 1651-1654 (1973) identified in the Kabat database as Kabpro: HKL111) and the BC2 light chain CDRs described previously. Five amino acid consensus human substitutions were introduced. Six framework amino
30 acids murine substitutions which can influence CDR presentation were made. Two overlapping synthetic oligonucleotides were generated (SEQ ID NOs: 63 and 64) which, when annealed and extended, code for amino acids representing the light chain variable region (SEQ ID
35 NOs: 65 and 66). This synthetic gene was then amplified using PCR primers (SEQ ID NOs: 67 and 68), ligated into

the pCR2000 vector (TA cloning Kit, Invitrogen, Cat. No. K2000-01) and isolated from a *ScaI*, *SacII* restriction digest. A second DNA fragment coding for the campath signal sequence including the first two amino acids of the variable region (SEQ ID NO: 38) was made by PCR amplification of the the appropriate region of a construct encoding a humanized anti-Respiratory Syncytial Virus heavy chain (SEQ ID NO: 25) with two primers (SEQ ID NOs: 26 and 69) and digesting with the restriction enzymes *EcoRI* and *ScaI*. A third DNA fragment encoding the remainder of a human framework 4 (SEQ ID NO: 70) and having *SacII* and *NarI* cohesive ends was generated by annealing two synthetic oligonucleotides (SEQ ID NOs: 71 and 72). F9HZLC 1-0 (SEQ ID NO: 43) was digested with the restriction enzymes *EcoRI* and *NarI* and ligated to the three DNA fragments. The sequence of the F9HZLC 2-0 insert is shown in SEQ ID NOs: 73 and 74.

SB 257731

SB 257731 contains the heavy chain F9HZHC 1-1 (SEQ ID NO: 52) and the light chain F9HZLC 1-3, a single amino acid mutation of F9HZLC 1-2 (SEQ ID NO: 62). F9HZLC 1-2 was PCR amplified with two primers (SEQ ID NOs: 26 and 69) and digested with the restriction enzymes *EcoRI* and *ScaI*. A 94 bp fragment (SEQ ID NOs: 75 and 76) was isolated. The fragment was ligated into *EcoRI*, *ScaI* digested F9HZLC 1-2 vector to produce the light chain construct F9HZLC 1-3. The sequence of the F9HZLC 1-3 insert is shown in SEQ ID NOs: 77 and 78.

SB 257732

SB 257732 contains the synthetic variable region humanized heavy chain F9HZHC 3-0 and light chain F9HZLC 3-0. Four overlapping synthetic oligonucleotides were generated (SEQ ID NOs: 79, 80, 81 and 82) which, when annealed and extended, code for the amino acids representing the heavy chain variable region being

altered (SEQ ID NOs: 83 and 84). This synthetic gene was then amplified using PCR primers (SEQ ID NOs: 85 and 86), ligated into the pCR2000 vector (TA cloning Kit, Invitrogen, Cat. No. K2000-01) and isolated from a *Stu*I, *Kpn*I restriction digest. The isolated fragment was ligated into *Stu*I, *Kpn*I digested F9HZHC1-1 (SEQ ID NO: 52) vector. This vector was then digested with *Eco*RI, *Spe*I to remove the signal sequence. A DNA fragment coding for the campath signal sequence (SEQ ID NO: 23) including the first five amino acids of the variable region was made by PCR amplification of F9HZHC1-0 with two primers (SEQ ID NOs: 26 and 87) and digesting with the restriction enzymes *Eco*RI and *Spe*I. The fragment generated was ligated into the vector. The sequence of the F9HZHC3-0 insert is shown in SEQ ID NOs: 88 and 89.

Four overlapping synthetic oligonucleotides were generated (SEQ ID NOs: 90, 91, 92 and 93) which, when annealed and extended, code for amino acids representing the light chain variable region (SEQ ID NOs: 94 and 95). This synthetic gene was then amplified using PCR primers (SEQ ID NOs: 96 and 97) and ligated into the pCR2000 vector (TA cloning Kit, Invitrogen, Cat. No. K2000-01), and isolated from a *Sca*I, *Nar*I restriction digest. The isolated fragment was ligated into *Sca*I, *Nar*I digested F9HZLC1-3 (SEQ ID NO: 77) vector. The sequence of the F9HZLC3-0 insert is shown in SEQ ID NOs: 98 and 99.

The humanized anti-Factor IX mAbs were expressed in CHO cells. A DG-44 cell line adapted for suspension growth in serum-free medium was grown in 100ml of protein-free medium containing 1X nucleosides and 0.05% F68 in 250 ml disposable sterile erlenmeyer flasks (Corning) on a Innova 2100 platform shaker (New Brunswick Scientific) at 150 rpm at 37°C in a 5% CO₂, 95% air humidified incubator. These cells were passaged at 4 X 10⁵ cells/ml twice weekly. 15 ug each of the pCN-Lc-Light Chain and pCD-Hc-heavy chain vectors were

linearized by digestion with *Not*I, co-precipitated under sterile conditions and resuspended in 50ul of 1X TE buffer (10mM Tris, 1mM EDTA, pH 7.5). The DNA was electroporated using a Bio-Rad Gene Pulser (Bio-Rad Laboratories) into the Acc-098 cells using the technique of Hensley *et al.* in *J. Biol. Chem.* 269, 23949-23958 (1994). 1.2×10^7 cells were washed once in 12.5 ml of ice cold PBSucrose (PBS, 272mM sucrose, 7mM sodium phosphate pH 7.4, 1mM $MgCl_2$), resuspended in 0.8 ml of PBS, added to 50ul of the DNA solution and incubated on ice for 15 min. The cells were pulsed at 380 V and 25 microfarads, then incubated on ice for 10 min. Cells were plated into 96 well culture plates at 5×10^5 cells/plate in maintenance medium for 24 hr prior to selection. Cells were selected for resistance to 400ug/ml G418 (Geneticin, Life Technologies, Inc.) in maintenance medium. 24 hr prior to assay, the cells were fed with 150ul of the maintenance medium.

Conditioned medium from individual colonies was assayed using an electrochemiluminescence (ECL) detection method on an Origen analyzer (IGEN, Inc.). See Yang *et al.*, *Biotechnology*, 12, 193-194 (1994).

All solutions necessary for the performance of the assays (assay buffer) and for the operation of the analyzer (cell cleaner) were obtained from IGEN. The antibodies (anti-human IgG (g-chain specific), Sigma Chemicals and F(ab')₂ Fragment to Human IgG (H+L), Kirkegaard & Perry Laboratories Inc.) were labelled with TAG-NHS-ester (IGEN, Inc.) at a 7:1 molar ratio of TAG:protein, while the Protein A (Sigma) was labelled with Biotin-LC-Sulfo-NHS-ester (IGEN, Inc.) at a 20:1 molar ratio Biotin:protein, both according to IGEN's recommendations. Streptavidin-coated magnetic beads (M-280) were obtained from Dynal.

Immunoassays were performed using the following protocol: per sample, 50ul of the Streptavidin-coated

beads (final concentration 600 ug/ml diluted in PBS, pH7.8, with 1.25% Tween) were mixed with 50ul Biotin-Protein A (final concentration 1ug/diluted in PBS, pH7.8, with 1.25% Tween) and incubated at room

5 temperature for 15min with agitation, 50ul of the TAG antibodies (a mixture with a final concentration of 1.25 ug/ml F(ab')₂ Fragment to Human IgG (H+L) and 0.25 ug/ml Anti-Human IgG (g-chain specific) diluted in PBS, pH7.8, with 1.25% Tween) were added, the solution was then
10 added to 50ul of conditioned medium and incubated with agitation at room temperature for 1 hr. 200ul of assay buffer was added to the reaction mix and the sample analyzed on the Origen I analyzer to measure ECL. The results indicated that approximately 20-37% of the
15 colonies assayed secrete over 15 ng/ml of the antibody with an average expression of about 150 ng/ml.

Humanized anti-Factor IX mAbs were purified from the conditioned media using a Procep A capture step followed by ion-exchange chromatography to reduce the
20 DNA burden. Procep A sorbent material (Bioprocessing Ltd., Durham, England) was used to prepare a column with a 1:1 diameter to height ratio. Clarified conditioned media was loaded onto the column at about 150 cm/hr. The column was washed sequentially with phosphate
25 buffered saline (PBS), PBS containing 1 M NaCl, and finally with PBS. The bound material was recovered with 0.1 M acetic acid elution. The eluate was adjusted to pH 5.5 and was diluted (1:4) with water. The diluted solution was loaded onto an S-Sepharose column (2.5 x 13
30 cm) which was pre-equilibrated with 20 mM sodium acetate, pH 5.5 at 80 cm/hr. The column was washed with the acetate buffer until a steady baseline was obtained and the bound protein was eluted with 20 mM sodium phosphate, pH 7.4 at 25 cm/hr. The eluted material was
35 filtered with a 0.4 micron membrane and stored at 4°C.

Example 7**Mouse-Human Chimeric Antibody**

100 ng of BC2 RNA were reverse transcribed with a RT-PCR kit per the manufacturer's instructions

5 (Boehringer Mannheim Cat. No. 1483-188) using a dT oligo for priming, and PCR amplified with synthetic *ScaI* (SEQ ID NO: 100) and *NarI* (SEQ ID NO: 101) primers to produce the BC2 light chain variable region with *ScaI*, *NarI* ends (SEQ ID NOs: 102 and 103). This DNA was ligated into
10 *ScaI*, *NarI* digested F9HZHC1-3 (SEQ ID 77) and digested with *ScaI*, *NarI* to produce a mouse-human chimeric light chain F9CHLC (SEQ ID NOs: 104 and 105).

100 ng of BC2 RNA were reverse transcribed with a RT-PCR kit per the manufacturer's instructions

15 (Boehringer Mannheim Cat. No. 1483-188) using a dT oligo for priming, and PCR amplified with synthetic *SpeI* (SEQ ID NO: 106) and *NheI* (SEQ ID NO: 107) primers to produce the BC2 heavy chain variable region with *SpeI*, *NheI* ends (SEQ ID NOs: 108 and 109). The campath signal sequence
20 was PCR amplified from the RSVHZ19 heavy chain (SEQ ID NO: 25) with *EcoRI* (SEQ ID 26) and *SpeI* (SEQ ID 87) primers. These two DNA fragments were ligated into a *EcoRI*, *NheI* digested IL4CHHCpcd vector described in published International Patent Application No.
25 WO95/07301, replacing the IL4 variable region with the BC2 Factor IX mouse variable region, to produce a mouse-human chimeric heavy chain F9CHHC (SEQ ID Nos: 110 and 111).

Co-transfection and purification of the mouse-human
30 chimeric antibody ch α FIX was accomplished as described above for the humanized constructs.

Example 8**Efficacy of humanized Factor IX mAbs in Rat Thrombus****Model**

In order to evaluate the efficacy of humanized
5 anti-Factor IX antibodies in prevention of arterial
thrombosis, the rat carotid artery thrombosis model as
described above in Example 3 was used. Baseline
parameters were established for carotid blood flow,
arterial pressure, heart rate, vessel patency and
10 activated partial thromboplastin time (aPTT). Fifteen
minutes thereafter, carotid injury was effected for 10
minutes. The parameters were determined 60 minutes
after onset of carotid injury. Carotid thrombus was
also extracted from the carotid artery and weighed.

15 All agents were administered intravenously 15
minutes before the onset of carotid injury. The
following treatments were examined and compared to the
anti-Factor IX mAb BC2.

1. Vehicle
- 20 2. ch α FIX: 3 mg/kg bolus
3. SB 249413: 3 mg/kg bolus
4. SB 249415: 3 mg/kg bolus
5. SB 249416: 3 mg/kg bolus
6. SB 249417: 3 mg/kg bolus
- 25 7. SB 257731: 3 mg/kg bolus
8. Heparin: 60 units/kg bolus + 2 units/kg/min
infusion

The aPTT was used as the primary criterion for
30 evaluation of efficacy versus bleeding liabilities of
the anti-coagulant/thrombotic agents used in the study.
The results in Fig. 8 demonstrate that the humanized
Factor IX mAbs SB 249413, SB 249415, SB 249416, SB
249417 and SB 257731 had a modest effect on aPTT at 3.0
35 mg/kg which is within the clinical accepted range.

The effect of the Factor IX mAbs on thrombus mass is shown in Fig. 9. The results indicate that all of the humanized mAbs are equally effective in reducing thrombus mass.

5 The studies conducted in the rat carotid thrombosis model clearly demonstrate the efficacy of the humanized Factor IX mAbs in prevention of thrombosis in a highly thrombogenic arterial injury model. Most notably, the efficacy of all of the humanized Factor IX mAbs was
10 demonstrated within the desired therapeutic anticoagulant target defined by the aPTT.

Example 9

Antibody Biochemical and Biophysical Properties

15 The molecular mass of SB 249417 was determined by MALD-MS to be 148,000Da. Analytical ultracentrifugation of SB 249417 gave an identical value. In the presence of Factor IX plus Ca^{2+} , the antibodies derived from BC 2 sedimented with a mass of 248,000Da corresponding to the
20 combined mass of the mAb and two molecules of Factor IX. No evidence of higher ordered aggregates was observed in the presence or absence of Factor IX.

The kinetics of Factor IX binding to SB 249417 was assessed by BIAcore analysis with antibody bound to an
25 immobilized protein A surface. Recombinant human Factor IX (rhFIX, Genetics Institute) at 49 nM was used and measurements performed in the presence of 5 mM Ca^{2+} . The interaction was characterized by rapid association, $k_{\text{ass}} = 2.0 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ and relatively slow off-rate, $k_{\text{diss}} = 4.1 \times 10^{-4} \text{ s}^{-1}$. The calculated K_d for Factor IX
30 binding was 1.9 nM.

Table 1 summarizes the biophysical properties of SB 249417.

35

Table 1

Summary of the Biophysical Properties of SB 249417

5	Isotype	IgG1, kappa
	Purity by SDS-PAGE	>95% (under reducing conditions)
	Molecular Weight	
10	Mass Spectrometry	148,000 Da
	Analytical Ultracentrifugation	148,000 Da
	Stoichiometry of Factor IX Binding	
15	IX: 1 mole mAb Isothermal Titration Calorimetry	1.5 moles Factor
	Factor IX Binding Affinity	
	Isothermal Titration Calorimetry	Kd= 4 nM at 25°C
20	Biosensor	Kd= 2 nM
	Factor IX Binding Kinetics	
	Biosensor	$k_{\text{ass}} = 2.0 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ $k_{\text{diss}} = 4 \times 10^{-4} \text{ s}^{-1}$
25	Table 2 summarizes the factor IX binding properties of mAbs of the present invention. The calculated dissociation constants were essentially identical within experimental error.	

Table 2

Kinetics of Factor IX Binding to Anti-Factor IX mAbs

<u>mAb</u>	<u>k_{ass} (M⁻¹s⁻¹)</u>	<u>k_{diss} (s⁻¹)</u>	<u>calc. K_D (nM)</u>
SB 249417	2.0 x10 ⁵	4.1x10 ⁻⁴	1.9
BC2	4.8x10 ⁵	9.1x10 ⁻⁴	1.9
Chf9	2.4 x10 ⁵	3.0x10 ⁻⁴	1.3
SB 249413	6.5 x10 ⁵	2.8x10 ⁻³	3.7-5.1
SB 249415	7.5 x10 ⁵	1.8x10 ⁻⁴	1.1-2.3
SB 249416	5.2 x10 ⁵	4.1x10 ⁻⁴	0.8
SB 257731	9.2 x10 ⁵	9.9x10 ⁻⁴	1.1
SB 257732	1.1 x10 ⁶	1.2x10 ⁻³	1.5

5

The interactions between rhFIX and SB 249417 , BC2 and other humanized constructs were characterized by titration microcalorimetry, which measures binding interactions in solution from the intrinsic heat of binding. Nine injections of 106 uM FIX were made into the calorimeter containing 2 uM mAb SB 249417. Binding was detected in the first 4 injections as exothermic heats. At the last 5 injections the mAb binding sites were saturated with FIX and only background heats of mixing were observed. The results indicated that the equivalence point occurred at a molar binding ratio near 2 FIX per mAb, as expected. Nonlinear least squares analysis of the data yield the binding affinity.

The rhFIX affinities of the mAbs were measured over a range of temperature from 34-44°C in 10mM HEPES, 10mM CaCl₂, 150mM NaCl, pH 7.4. These data allow the affinity at 37°C to be determined directly and the affinity at 25°C to be calculated from the van't Hoff

equation. The data in Table 3 indicate that the affinities of SB 249417, BC2 and its other humanized constructs are within error (a factor of 2) the same.

Table 3

5 Titration Calorimetry Results for Anti-FIX mAbs

mAb	Kd, nM at 25°C	Kd, nM at 37°C	Molar Binding Ratio FIX/mAb
BC2	10	20	1.4
SB 249413	6	12	1.9
SB 249415	3	7	1.7
SB 249417	4	12	1.5
SB 257732	4	9	1.8

The mAbs SB 249413, SB 249415, SB 249417 and SB 257732 all exhibited very similar thermal stabilities by differential scanning calorimetry. Their unfolding Tms ranged from 70-75°C indicating high stability against thermally induced denaturation.

Example 10

15 Mechanism of Antibody-Mediated Inhibition of Factor IX

A library of chimeric constructs composed of sequences of Factor IX spliced into the framework of the homologous protein Factor VII was constructed and used to map the epitope for the Factor IX BC2 mAb. See
 20 Cheung et al., *Thromb. Res.* 80, 419-427 (1995). Binding was measured using a BiaCore 2000 surface plasmon

resonance device. The BC2 antibody was coupled directly to the chip using the NHS/EDC reaction. Binding was measured by 2 min of contact time at 20uL/min with 200 nM of each of the given constructs in 25 mM MOPS, pH 7.4, 0.15 M NaCl, 5 mM CaCl₂. Dissociation was monitored for 3 min using the same buffer with no protein. No binding was detected to the wild type construct in the presence of 50 mM EDTA. The data are presented in Table 4.

10

Table 4

Summary of Binding of Factor IX Constructs to BC2 Antibody

Construct	Degree of Binding
Plasma IXa	Binds
r-IX	Binds
Plasma VII	No Binding
IX LC/VII HC	Binds
IX-A/VII	Binds
VII gla/IX	No Binding
VII-A/IX	No Binding
VII gla (IX 3-11)/IX	Binds
VII gla (IX 3-6)/IX	Very Low Binding
VII gla (IX 9-11)/IX	Very Low Binding
IX K5A	Binds

15

These data indicate that the constructs containing the Factor IX light chain and Factor VII heavy chain (IX LC/VII HC); the Factor IX gla and aromatic stack domains (IX-A/VII); residues 3-11 of Factor IX gla domain within

the Factor VII gla domain (VII gla (IX 3-11)/IX); and Factor IX having a lysine to alanine substitution at residue 5 (IX K5A) exhibit binding to BC2. The VII gla (IX 3-11)/IX construct exhibited BC2 binding equivalent to wild type Factor IX (plasma IXa and r-IX). Thus, the BC2 antibody binds to an epitope contained within residues 3-11 of the Factor IX gla domain.

Example 11

Treatment of Arterial Thrombosis with Anti-Factor IX Antibody and Tissue Plasminogen Activator

Administration of tPA with or without adjunctive therapies, was initiated following complete occlusion of the carotid artery. Blood flow in the artery was continuously monitored.

Male Sprague-Dawley rats (Charles River, Raleigh, NC) weighing 300-490 gm were anesthetized with sodium pentobarbital (55 mg/kg, i.p.). The rats were placed dorsal on a heated (37°C) surgical board and an incision was made in the neck; the trachea was isolated and cannulated with a PE-240, Intramedic tube. The left carotid artery and jugular vein were then isolated. A Parafilm M sheet (4 mm², American National Can) was placed under the carotid artery and an electromagnetic blood flow probe (Carolina Medical) was placed on the artery to measure blood flow. A cannula (Tygon, 0.02" x 0.04", Norton Performance Plastics) was inserted into the jugular vein for drug administration. The left femoral artery was then isolated and cannulated for measurement of blood pressure and collection of blood samples.

Thrombosis in the carotid artery was initiated with a 6.5 mm diameter circular patch of glass micro-filter paper saturated with FeCl₃ solution (50%) placed on the carotid artery downstream from the flow probe for 10 minutes as described in Example 3. In this well-

characterized model, thrombus formation is usually complete within 15 min.

The anti-Factor IX antibody, SB 249415 was administered as a bolus in combination with tPA (Genentech, South San Francisco, CA), while heparin (Elkins-Sinn Inc., Cherry Hill, NJ) was administered as a bolus followed by infusion. All drug infusions continued to the end of the experimental period - 60 minutes from the time of vessel occlusion. Blood samples, 1 mL, were collected for aPTT and PT assay at 0, 30 and 60 min (end of study) from the femoral artery into 3.8% citrate solution and centrifuged. aPTT and prothrombin time (PT) were monitored by a fibrometer (BB1L, Baxter Dade or MLA Electra 800 Automatic Coagulation Timer) with standard procedures. At the end of the experiment, the thrombus was extracted from the carotid artery and weighed.

All data are presented as mean group values \pm SEM for the indicated number of rats in each group. ANOVA and Bonferoni tests for multiple comparisons were used for between group analyses and a $p < 0.05$ accepted as significance.

Formation of an occlusive thrombus occurs approximately 15 min after initiation of arterial injury by application of the FeCl_3 treated patch to the rat carotid artery. As shown in Fig. 10, with tPA alone, reperfusion of the occluded vessel was only observed following administration of a dose of 9 mg/kg tPA with 67% of the treated vessels exhibiting regain of blood flow during the 60 min protocol. At this dose of tPA inclusion of 60 U/kg heparin or 3 mg/kg anti-Factor IX antibody, SB 249415, did not result in a further increase in the incidence of reperfusion suggesting that in the FeCl_3 injury model about 30% of the thrombi are refractory to lysis.

The results in Fig. 10 indicate that, at lower doses of tPA, the incidence of reperfusion is significantly dependent upon which anticoagulant was co-administered with the thrombolytic. When 60 U/kg heparin was administered the percentage of vessels showing reperfusion decreased dramatically with only 12.5% and 40% reperfusion observed with 3 and 6 mg/kg tPA, respectively. Co-administration of 3 mg/kg SB 249415 with the tPA, however, achieved greater than 60% reperfusion with 3 mg/kg tPA and 79% reperfusion with the 6 mg/kg tPA dose. Thus, the anti-Factor IX antibody significantly shifts the thrombolytic dose response curve allowing reperfusion with lower doses of thrombolytic agent.

Thrombolysis and clot formation are dynamic processes and periods of patency followed by re-occlusion were sometimes observed. Since carotid blood flow was monitored continuously during the 60 min experimental protocol, it was also possible to quantitate the total time of carotid patency. As shown in Fig. 11, the total period of vessel patency is substantially increased by combination of 3 mg/kg anti-Factor IX antibody plus tPA. This is particularly evident at the lowest and the intermediate doses of tPA, 3 and 6 mg/kg, respectively. At a combined dose of 3 mg/kg SB 249415 plus 3 mg/kg tPA, the total patency time was 30.6 ± 9.2 min compared to 7.1 ± 7.1 min for the combination of 60 U/kg heparin plus 3 mg/kg tPA. Patency time was zero with 3 mg/kg tPA alone. With a dose of 6 mg/kg tPA, co-administration with heparin increases patency time only slightly to 12.9 ± 6.0 min whereas the tPA-SB 249415 combination achieves maximal patency time of 38.7 ± 8.4 min. Only at the highest dose of tPA (9 mg/kg) does the heparin combination approach the patency achieved with SB 249415, 31.9 ± 4.8 min and 38.0 ± 8.4 min, respectively.

Rapid restoration of blood flow following arterial infarct is critical to minimizing damage to the ischemic tissue. The results in Fig. 12 indicate that the combination of anti-factor IX antibody with tPA resulted in decreased time to reperfusion compared to tPA alone or heparin plus tPA and that this is achieved with lower doses of tPA. When thrombolysis was effected with 3 mg/kg SB 249415 plus 3 mg/kg tPA the time to thrombolysis was 29.4 ± 9.2 min. With 3 mg/kg tPA, alone, no reperfusion was observed. With 60 U/kg heparin plus 3 mg/kg tPA the time to thrombolysis was 52.8 ± 7.1 min. At higher doses of tPA, 6 and 9 mg/kg, the antibody plus tPA treatment regimen achieved initial thrombolysis in 19.4 ± 6.3 and 20.8 ± 8.7 min, respectively. In the absence of added anticoagulant, the time to thrombolysis was 60 min (i.e., the limit of the experimental protocol) and 27.5 ± 6.4 min for doses of 6 and 9 mg/kg, respectively. With addition of 60 U/kg heparin, the corresponding times to thrombolysis were 44.0 ± 7.1 and 27.0 ± 4.9 min. Thus, earlier reperfusion was always achieved with SB 249415 than with heparin or with tPA alone.

Example 12

Effect of Anti-Factor IX Antibody on Hemostatic Function

The impact of anti-factor IX or heparin as adjunctive agents on the maintenance of hemostatic function was determined by monitoring levels of fibrinogen, plasminogen and alpha-2-antiplasmin at the end of the treatment period in rats treated with tPA alone, tPA plus heparin and tPA plus SB 249415 and the results were compared to vehicle treated animals. As shown in Fig. 13, increasing doses of tPA resulted in decreased levels of each of the hemostatic markers measured. Alpha-2-antiplasmin levels dropped from about 90% in animals not treated with tPA to about 20% as the

dose of tPA was increased to 9 mg/kg. Plasminogen levels dropped from an average of about 100% without tPA treatment to about 40% in the 9 mg/kg treatment group. Likewise, fibrinogen levels dropped from about 150 mg/dL to about 90 mg/dL in the high-dose tPA group. Interestingly, the selection of the adjunctive agent does not appear to significantly effect any of these markers. At each tPA dose, similar levels of alpha-2-antiplasmin, plasminogen and fibrinogen were observed in animals given vehicle, 30 or 60 U/kg heparin or 1 or 3 mg/kg SB 249415; the observed decrease in the hemostatic markers is only a function of dose of the thrombolytic agent, tPA, and these decreases, especially in the case of fibrinogen, appear to be particularly large with tPA doses greater than 6 mg/kg, i.e., in the 9 mg/kg high-dose group.

The effects of the different treatment regimens on the standard aPTT coagulation assay aPTT was also monitored (Fig. 14). With increasing doses of tPA the aPTT increased from 19.3s \pm 0.6s to 30.0s \pm 1.6s for vehicle and 9 mg/kg tPA, respectively. Administration of 3 mg/kg SB 249415 produced a limited increase in the aPTT of control animals to 49.6 s \pm 6.4 s. When SB 249415 was co-administered with tPA the observed increase was slightly larger and was dependent upon the dose of tPA. Combination of SB 249415 with 3 mg/kg tPA produced an aPTT of 58.3 s \pm 5.2 s whereas combination of SB 249415 with the 9 mg/kg dose of tPA increased the aPTT to 77.3 s \pm 19.7 s. Administration of either the 30 U/kg or 60 U/kg dose of heparin resulted in large increases in the aPTT. Without tPA the aPTT ranged from about 300 s to 600 s for 30 and 60 U/kg doses, respectively. In tPA treated animals the aPTT was about 800 s.

The elevation of the aPTT obtained with heparin, particularly when coupled with the perturbation of

hemostatic parameters due to the need for high doses of tPA to achieve effective reperfusion, is likely to contribute to bleeding liabilities. Conversely, SB 249415 does not cause major elevation of the aPTT and enables the use of lower doses of tPA providing significant advantage in thrombolytic therapy in myocardial infarction and stroke.

The present invention may be embodied in other specific forms without departing from the spirit or essential attributes thereof, and, accordingly, reference should be made to the appended claims, rather than to the foregoing specification, as indicating the scope of the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION

- (i) APPLICANT: Blackburn, Michael
Feuerstein, Giora
Patel, Arunbhai
- (ii) TITLE OF INVENTION: ANTICOAGULANT AGENTS USEFUL IN
TREATMENT OF THROMBOSIS
- (iii) NUMBER OF SEQUENCES: 111
- (iv) CORRESPONDENCE ADDRESS:
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 - (C) CITY: King of Prussia
 - (D) STATE: PA
 - (E) COUNTRY: USA
 - (F) ZIP: 19406
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Diskette
 - (B) COMPUTER: IBM Compatible
 - (C) OPERATING SYSTEM: DOS
 - (D) SOFTWARE: FastSEQ Version 1.5
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: 09/346,487
 - (B) FILING DATE: 01-JUL-1999
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: 08/783,853
 - (B) FILING DATE: 16-JAN-1997
- (viii) ATTORNEY/AGENT INFORMATION:
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 - (C) REFERENCE/DOCKET NUMBER: P50438-1

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- (B) TELEFAX:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CATCCTAGAG TCACCGAGGA

20

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

AGCTGCCCAA AGTGCCCAAG C

21

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CTAACTCA TTCCTGTTGA AGCTCTTGAC AATGGG

36

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GATTTTCARG TGCAGATTTT C

21

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 363 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO
 (v) FRAGMENT TYPE:
 (vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CAGATCCAGT	TGGTGCAGTC	TGGACCTGAG	CTGAAGAAGC	CTGGAGAGAC	AGTCAAGATC	60
TCCTGCAAGG	CTTCTGGGTA	CACCTTCACA	AACTATGGAA	TGAACTGGGT	GAAGCAGGCT	120
CCAGGAAAGG	GTTTAAAGTG	GATGGGCTGG	ATAAACACCA	GAAATGGAAA	GTCAACATAT	180
GTTGATGACT	TCAAGGGACG	GTTTGCCTTC	TCTTTGGAAA	GCTCTGCCAG	CACTGCCAAT	240
TTGCAGATCG	ACAACCTCAA	AGATGAGGAC	ACGGCTACAT	ATTTCTGTAC	AAGAGAAGGG	300
AATATGGATG	GTTACTTCCC	TTTTACTTAC	TGGGGCCAAG	GGACTCTGGT	CACTGTCTCT	360
GCA						363

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 321 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CAAATTGTTC	TCTCCCAGTC	TCCAGCAATC	CTGTCTGCAT	CTCCAGGGGA	GAAGGTCACA	60
ATGACTTGCA	GGGCCAGCTC	AAGTGTAAT	TACATGCACT	GGTACCAGCA	GAAGCCAGGA	120
TCCTCCCCCA	AACCCTGGAT	TTATGCCACA	TCCAACCTGG	CTTCTGGAGT	CCCTGCTCGC	180
TTCAGTGGCA	GTGGGTCTGG	GACCTCTTAC	TCTCTCACAA	TCAGCAGAGT	GGAGGCTGAA	240
GATGCTGCCA	CTTATTACTG	CCAGCAGTGG	AGTATTAACC	CACGGACGTT	CGGTGGAGGC	300
ACCAAGCTGG	AAATCAAACG	G				321

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 121 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
 (iii) HYPOTHETICAL: NO
 (iv) ANTISENSE: NO
 (v) FRAGMENT TYPE: internal
 (vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Gln	Ile	Gln	Leu	Val	Gln	Ser	Gly	Pro	Glu	Leu	Lys	Lys	Pro	Gly	Glu
1				5					10					15	
Thr	Val	Lys	Ile	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Asn	Tyr
			20					25					30		
Gly	Met	Asn	Trp	Val	Lys	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Lys	Trp	Met
		35					40					45			
Gly	Trp	Ile	Asn	Thr	Arg	Asn	Gly	Lys	Ser	Thr	Tyr	Val	Asp	Asp	Phe
		50				55				60					
Lys	Gly	Arg	Phe	Ala	Phe	Ser	Leu	Glu	Ser	Ser	Ala	Ser	Thr	Ala	Asn
65				70					75					80	
Leu	Gln	Ile	Asp	Asn	Leu	Lys	Asp	Glu	Asp	Thr	Ala	Thr	Tyr	Phe	Cys
			85					90						95	
Thr	Arg	Glu	Gly	Asn	Met	Asp	Gly	Tyr	Phe	Pro	Phe	Thr	Tyr	Trp	Gly
		100					105						110		
Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ala							
		115					120								

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 5 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
 (iii) HYPOTHETICAL: NO
 (iv) ANTISENSE: NO
 (v) FRAGMENT TYPE: internal
 (vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Asn	Tyr	Gly	Met	Asn
1				5

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Trp	Ile	Asn	Thr	Arg	Asn	Gly	Lys	Ser	Thr	Tyr	Val	Asp	Asp	Phe	Lys
1				5					10					15	
Gly															

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Glu	Gly	Asn	Met	Asp	Gly	Tyr	Phe	Pro	Phe	Thr	Tyr
1				5						10	

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 107 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
 (iii) HYPOTHETICAL: NO
 (iv) ANTISENSE: NO
 (v) FRAGMENT TYPE: internal
 (vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Gln	Ile	Val	Leu	Ser	Gln	Ser	Pro	Ala	Ile	Leu	Ser	Ala	Ser	Pro	Gly
1				5					10					15	
Glu	Lys	Val	Thr	Met	Thr	Cys	Arg	Ala	Ser	Ser	Ser	Val	Asn	Tyr	Met
			20					25					30		
His	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Ser	Ser	Pro	Lys	Pro	Trp	Ile	Tyr
		35				40						45			
Ala	Thr	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro	Ala	Arg	Phe	Ser	Gly	Ser
		50				55					60				
Gly	Ser	Gly	Thr	Ser	Tyr	Ser	Leu	Thr	Ile	Ser	Arg	Val	Glu	Ala	Glu
65					70					75					80
Asp	Ala	Ala	Thr	Tyr	Cys	Gln	Gln	Trp	Ser	Ile	Asn	Pro	Arg	Thr	
			85					90					95		
Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg					
			100					105							

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
 (iii) HYPOTHETICAL: NO
 (iv) ANTISENSE: NO
 (v) FRAGMENT TYPE: internal
 (vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Arg Ala Ser Ser Ser Val Asn Tyr Met His
1 5 10

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Ala Thr Ser Asn Leu Ala Ser
1 5

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Gln Gln Trp Ser Ile Asn Pro Arg Thr
1 5

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

```
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
```

CAACTAGTGC	AATCTGGGTC	TGAGTTGAAG	AAGCCTGGGG	CCTCAGTGAA	GGTTTCCTGC	60
AAGGCCCTCTG	GATACACCTT	CACTAACTAT	GGAATGAACT	GGGT		104

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 108 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TTGAAGTCAT	CAACATATGT	TGACTTTTCCA	TTTCTGGTGT	TTATCCATCC	CATCCACTCG	60
AGCCCTTGTG	CAGGGGCCTG	TCGCACCCAG	TTCATTCCAT	AGTTAGTG		108

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 107 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

76

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

(2) INFORMATION FOR SEQ ID NO:18:

```
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
```

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 337 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(ix) FEATURE:
```

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

(2) INFORMATION FOR SEQ ID NO:20:

78

(ii) MOLECULE TYPE: protein
 (iii) HYPOTHETICAL: NO
 (iv) ANTISENSE: NO
 (v) FRAGMENT TYPE: internal
 (vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Leu	Val	Gln	Ser	Gly	Ser	Glu	Leu	Lys	Lys	Pro	Gly	Ala	Ser	Val	Lys
1				5					10					15	
Val	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Asn	Tyr	Gly	Met	Asn
			20					25					30		
Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Met	Gly	Trp	Ile
			35				40					45			
Asn	Thr	Arg	Asn	Gly	Lys	Ser	Thr	Tyr	Val	Asp	Asp	Phe	Lys	Gly	Arg
			50			55					60				
Phe	Val	Phe	Ser	Leu	Asp	Thr	Ser	Val	Ser	Thr	Ala	Tyr	Leu	Gln	Ile
65				70					75					80	
Ser	Ser	Leu	Lys	Ala	Asp	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	Ala	Arg	Glu
			85					90					95		
Gly	Asn	Met	Asp	Gly	Tyr	Phe	Pro	Phe	Thr	Tyr	Trp	Gly	Gln	Gly	Thr
			100					105					110		

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
 (iii) HYPOTHETICAL: NO
 (iv) ANTISENSE: NO
 (v) FRAGMENT TYPE:
 (vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GCTACTAGTG CAATCTGGGT CTGAGTTGAA GCC

33

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTISENSE: NO
- (v) FRAGMENT TYPE:
- (vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

TGGGTACCCT GGCCCCAGTA AGTAAAAGGG

30

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 97 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTISENSE: NO
- (v) FRAGMENT TYPE:
- (vi) ORIGINAL SOURCE:
- (ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 27...95
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GAATTCTGAG CACACAGGAC CTCACC ATG GGA TGG AGC TGT ATC ATC CTC TTC
Met Gly Trp Ser Cys Ile Ile Leu Phe

53

1 5

TTG GTA GCA ACA GCT ACA GGT GTC CAC TCC CAG GTC CAA CTA GT
 Leu Val Ala Thr Ala Thr Gly Val His Ser Gln Val Gln Leu
 10 15 20

97

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
 1 5 10 15
 Val His Ser Gln Val Gln Leu
 20

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 110 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
GGAGACGCCA TCGAATTCTG AGCACACAGG ACCTCACCAT GGGATGGAGC TGTATCATCC 60
TCTTCTTGGT AGCAACAGCT ACAGGTGTCC ACTCCCAGGT CCAACTGCAG 110
```

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

```
GGAGACGCCA TCGAATTCTG A 21
```

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```
GATTGCACTA GTTGGACCTG GGAGTGGACA 30
```

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 77 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CTAGAGTGGG TCGCAGAGAT CTCTGATGGT GGTAGTTACA CCTACTATCC AGACACTGTG	60
ACGGGCCCGGT TCACGAT	77

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

ATCGTGAACC GGCCCGTCAC AGTGTCTGGA TAGTAGGTGT AACTACCACC ATCAGAGATC	60
TCTGCGACCC ACT	73

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 363 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence

(B) LOCATION: 1...363

(D) OTHER INFORMATION: F9HZHC 1-0

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

CAG GTG CAA CTA GTG CAA TCT GGG TCT GAG TTG AAG AAG CCT GGG GCC	48
Gln Val Gln Leu Val Gln Ser Gly Ser Glu Leu Lys Lys Pro Gly Ala	
1 5 10 15	
TCA GTG AAG GTT TCC TGC AAG GCC TCT GGA TAC ACC TTC ACT AAC TAT	96
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr	
20 25 30	
GGA ATG AAC TGG GTG CGA CAG GCC CCT GGA CAA GGG CTC GAG TGG ATG	144
Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met	
35 40 45	
GGA TGG ATA AAC ACC AGA AAT GGA AAG TCA ACA TAT GTT GAT GAC TTC	192
Gly Trp Ile Asn Thr Arg Asn Gly Lys Ser Thr Tyr Val Asp Asp Phe	
50 55 60	
AAG GGA CGG TTT GTC TTC TCC TTG GAC ACC TCT GTC AGC ACG GCA TAT	240
Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr	
65 70 75 80	
CTA CAG ATC AGC AGC CTA AAG GCT GAC GAC ACT GCA GTG TAT TAC TGT	288
Leu Gln Ile Ser Ser Leu Lys Ala Asp Asp Thr Ala Val Tyr Tyr Cys	
85 90 95	
GCG AGA GAA GGG AAT ATG GAT GGT TAC TTC CCT TTT ACT TAC TGG GGC	336
Ala Arg Glu Gly Asn Met Asp Gly Tyr Phe Pro Phe Thr Tyr Trp Gly	
100 105 110	

363

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 121 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- ```
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
```

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Val | Gln | Leu | Val | Gln | Ser | Gly | Ser | Glu | Leu | Lys | Lys | Pro | Gly | Ala |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |
| Ser | Val | Lys | Val | Ser | Cys | Lys | Ala | Ser | Gly | Tyr | Thr | Phe | Thr | Asn | Tyr |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |
| Gly | Met | Asn | Trp | Val | Arg | Gln | Ala | Pro | Gly | Gln | Gly | Leu | Glu | Trp | Met |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |
| Gly | Trp | Ile | Asn | Thr | Arg | Asn | Gly | Lys | Ser | Thr | Tyr | Val | Asp | Asp | Phe |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |
| Lys | Gly | Arg | Phe | Val | Phe | Ser | Leu | Asp | Thr | Ser | Val | Ser | Thr | Ala | Tyr |
| 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |
| Leu | Gln | Ile | Ser | Ser | Leu | Lys | Ala | Asp | Asp | Thr | Ala | Val | Tyr | Tyr | Cys |
|     |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |
| Ala | Arg | Glu | Gly | Asn | Met | Asp | Gly | Tyr | Phe | Pro | Phe | Thr | Tyr | Trp | Gly |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |
| Gln | Gly | Thr | Leu | Val | Thr | Val | Ser | Ser |     |     |     |     |     |     |     |
|     |     | 115 |     |     |     |     | 120 |     |     |     |     |     |     |     |     |

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 165 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA  
 (iii) HYPOTHETICAL: NO  
 (iv) ANTISENSE: NO  
 (v) FRAGMENT TYPE:  
 (vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

|                                                                   |     |
|-------------------------------------------------------------------|-----|
| AGTACTGACA CAGTCTCCAG CCACCCTGTC TTTGTCTCCA GGGGAAAGAG CCACCCTCTC | 60  |
| CTGCAGGGCC AGCTCAAGTG TAAATTACAT GCACTGGTAC CAACAGAGAC CTGGCCAGGC | 120 |
| TCCCAGGCTC CTCATCTATG CCACTAGTAA CCTGGCTTCT GGCAT                 | 165 |

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 146 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA  
 (iii) HYPOTHETICAL: NO  
 (iv) ANTISENSE: NO  
 (v) FRAGMENT TYPE:  
 (vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

|                                                                   |     |
|-------------------------------------------------------------------|-----|
| CCGCGGGTTA ATACTCCACT GCTGACAGTA ATAAACCGCA AAATCTTCAG GCTCTAGACT | 60  |
| GCTGATGGTG AGAGTGAAAT CTGTCCCAGA CCCGGATCCA CTGAACCTGG CTGGGATGCC | 120 |
| AGAAGCCAGG TTACTAGTGG CATAGA                                      | 146 |

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 280 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA  
 (iii) HYPOTHETICAL: NO  
 (iv) ANTISENSE: NO

- (v) FRAGMENT TYPE:  
 (vi) ORIGINAL SOURCE:  
 (ix) FEATURE:

- (A) NAME/KEY: Coding Sequence  
 (B) LOCATION: 2...280  
 (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

|   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| A | GTA | CTG | ACA | CAG | TCT | CCA | GCC | ACC | CTG | TCT | TTG | TCT | CCA | GGG | GAA | AGA | 49  |
|   | Val | Leu | Thr | Gln | Ser | Pro | Ala | Thr | Leu | Ser | Leu | Ser | Pro | Gly | Glu | Arg |     |
|   | 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |     |
|   | GCC | ACC | CTC | TCC | TGC | AGG | GCC | AGC | TCA | AGT | GTA | AAT | TAC | ATG | CAC | TGG | 97  |
|   | Ala | Thr | Leu | Ser | Cys | Arg | Ala | Ser | Ser | Ser | Val | Asn | Tyr | Met | His | Trp |     |
|   |     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |     |
|   | TAC | CAA | CAG | AGA | CCT | GGC | CAG | GCT | CCC | AGG | CTC | CTC | ATC | TAT | GCC | ACT | 145 |
|   | Tyr | Gln | Gln | Arg | Pro | Gly | Gln | Ala | Pro | Arg | Leu | Leu | Ile | Tyr | Ala | Thr |     |
|   |     |     |     | 35  |     |     |     | 40  |     |     |     |     | 45  |     |     |     |     |
|   | AGT | AAC | CTG | GCT | TCT | GGC | ATC | CCA | GCC | AGG | TTC | AGT | GGA | TCC | GGG | TCT | 193 |
|   | Ser | Asn | Leu | Ala | Ser | Gly | Ile | Pro | Ala | Arg | Phe | Ser | Gly | Ser | Gly | Ser |     |
|   |     |     |     | 50  |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |
|   | GGG | ACA | GAT | TTC | ACT | CTC | ACC | ATC | AGC | AGT | CTA | GAG | CCT | GAA | GAT | TTT | 241 |
|   | Gly | Thr | Asp | Phe | Thr | Leu | Thr | Ile | Ser | Ser | Leu | Glu | Pro | Glu | Asp | Phe |     |
|   | 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     | 80  |     |     |
|   | GCG | GTT | TAT | TAC | TGT | CAG | CAG | TGG | AGT | ATT | AAC | CCG | CGG |     |     |     | 280 |
|   | Ala | Val | Tyr | Tyr | Cys | Gln | Gln | Trp | Ser | Ile | Asn | Pro | Arg |     |     |     |     |
|   |     |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     |     |     |     |

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 93 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein

- (iii) HYPOTHETICAL: NO
- (iv) ANTISENSE: NO
- (v) FRAGMENT TYPE: internal
- (vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Leu | Thr | Gln | Ser | Pro | Ala | Thr | Leu | Ser | Leu | Ser | Pro | Gly | Glu | Arg |
| 1   |     |     |     | 5   |     |     |     | 10  |     |     |     |     | 15  |     |     |
| Ala | Thr | Leu | Ser | Cys | Arg | Ala | Ser | Ser | Ser | Val | Asn | Tyr | Met | His | Trp |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |
| Tyr | Gln | Gln | Arg | Pro | Gly | Gln | Ala | Pro | Arg | Leu | Leu | Ile | Tyr | Ala | Thr |
|     |     |     | 35  |     |     |     | 40  |     |     |     |     | 45  |     |     |     |
| Ser | Asn | Leu | Ala | Ser | Gly | Ile | Pro | Ala | Arg | Phe | Ser | Gly | Ser | Gly | Ser |
|     |     | 50  |     |     |     | 55  |     |     |     | 60  |     |     |     |     |     |
| Gly | Thr | Asp | Phe | Thr | Leu | Thr | Ile | Ser | Ser | Leu | Glu | Pro | Glu | Asp | Phe |
| 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     | 80  |     |
| Ala | Val | Tyr | Tyr | Cys | Gln | Gln | Trp | Ser | Ile | Asn | Pro | Arg |     |     |     |
|     |     |     |     | 85  |     |     |     | 90  |     |     |     |     |     |     |     |

(2) INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 27 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTISENSE: NO
- (v) FRAGMENT TYPE:
- (vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

TCGAGTACTG ACACAGTCTC CAGCCAC

27

(2) INFORMATION FOR SEQ ID NO:37:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 27 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GACCGCGGGT TAATACTCCA CTGCTGA

27

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 94 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence

(B) LOCATION: 27...92

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

|                                                                  |    |
|------------------------------------------------------------------|----|
| GAATTCTGAG CACACAGGAC CTCACC ATG GGA TGG AGC TGT ATC ATC CTC TTC | 53 |
| Met Gly Trp Ser Cys Ile Ile Leu Phe                              |    |
| 1 5                                                              |    |

|                                                        |    |
|--------------------------------------------------------|----|
| TTG GTA GCA ACA GCT ACA GGT GTC CAC TCC GAG ATA GTA CT | 94 |
| Leu Val Ala Thr Ala Thr Gly Val His Ser Glu Ile Val    |    |
| 10 15 20                                               |    |

(2) INFORMATION FOR SEQ ID NO:39:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Gly | Trp | Ser | Cys | Ile | Ile | Leu | Phe | Leu | Val | Ala | Thr | Ala | Thr | Gly |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |
| Val | His | Ser | Glu | Ile | Val |     |     |     |     |     |     |     |     |     |     |
|     |     |     |     | 20  |     |     |     |     |     |     |     |     |     |     |     |

## (2) INFORMATION FOR SEQ ID NO:40:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

GACTGTGTCA GTACTATCTC GGAGTGGACA

30

## (2) INFORMATION FOR SEQ ID NO:41:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 55 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA  
 (iii) HYPOTHETICAL: NO  
 (iv) ANTISENSE: NO  
 (v) FRAGMENT TYPE:  
 (vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

GGGCAGCCTC CTAAGTTGCT CATTTACTGG GCGTCGACTA GGGAATCTGG GGTAC

55

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 51 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA  
 (iii) HYPOTHETICAL: NO  
 (iv) ANTISENSE: NO  
 (v) FRAGMENT TYPE:  
 (vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

CCCAGATTCC CTAGTCGACG CCCAGTAAAT GAGCAACTTA GGAGGCTGCC C

51

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 321 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA  
 (iii) HYPOTHETICAL: NO  
 (iv) ANTISENSE: NO  
 (v) FRAGMENT TYPE:  
 (vi) ORIGINAL SOURCE:  
 (ix) FEATURE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

(2) INFORMATION FOR SEQ ID NO:44:

(A) LENGTH: 107 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

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(iii) HYPOTHETICAL: NO  
 (iv) ANTISENSE: NO  
 (v) FRAGMENT TYPE: internal  
 (vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Ile | Val | Leu | Thr | Gln | Ser | Pro | Ala | Thr | Leu | Ser | Leu | Ser | Pro | Gly |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |
| Glu | Arg | Ala | Thr | Leu | Ser | Cys | Arg | Ala | Ser | Ser | Ser | Val | Asn | Tyr | Met |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |
| His | Trp | Tyr | Gln | Gln | Arg | Pro | Gly | Gln | Ala | Pro | Arg | Leu | Leu | Ile | Tyr |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |
| Ala | Thr | Ser | Asn | Leu | Ala | Ser | Gly | Ile | Pro | Ala | Arg | Phe | Ser | Gly | Ser |
|     |     | 50  |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |
| Gly | Ser | Gly | Thr | Asp | Phe | Thr | Leu | Thr | Ile | Ser | Ser | Leu | Glu | Pro | Glu |
|     | 65  |     |     |     | 70  |     |     |     | 75  |     |     |     |     | 80  |     |
| Asp | Phe | Ala | Val | Tyr | Tyr | Cys | Gln | Gln | Trp | Ser | Ile | Asn | Pro | Arg | Thr |
|     |     |     | 85  |     |     |     |     | 90  |     |     |     | 95  |     |     |     |
| Phe | Gly | Gly | Gly | Thr | Lys | Val | Glu | Ile | Lys | Arg |     |     |     |     |     |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     |     |     |     |     |

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 134 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

|            |            |            |            |            |            |     |
|------------|------------|------------|------------|------------|------------|-----|
| CCTGGACAAG | GGCTCAAGTG | GATGGGATGG | ATAAACACCA | GAAATGGAAA | GTCAACATAT | 60  |
| GTTGATGACT | TCAAGGGACG | GTTTGTCTTC | TCTCTAGACT | CCTCTGTCAG | CACGGCATAT | 120 |
| CTACAGATCA | GCAG       |            |            |            |            | 134 |

(2) INFORMATION FOR SEQ ID NO:46:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 134 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

```

GGTACCCTGG CCCCAGTAAG TAAAAGGGAA GTAACCATCC ATATTCCTT CTCTCGTACA 60
GTAATACACT GCAGTGTCGT CAGCCTTTAG GCTGCTGATC TGTAGATATG CCGTGCTGAC 120
AGAGGAGTCT AGAG 134

```

## (2) INFORMATION FOR SEQ ID NO:47:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 225 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence

(B) LOCATION: 1...225

(D) OTHER INFORMATION:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

```

CCT GGA CAA GGG CTC AAG TGG ATG GGA TGG ATA AAC ACC AGA AAT GGA 48
Pro Gly Gln Gly Leu Lys Trp Met Gly Trp Ile Asn Thr Arg Asn Gly
1 5 10 15

```

AAG TCA ACA TAT GTT GAT GAC TTC AAG GGA CGG TTT GTC TTC TCT CTA 96  
 Lys Ser Thr Tyr Val Asp Asp Phe Lys Gly Arg Phe Val Phe Ser Leu  
                   20                                  25                                  30

GAC TCC TCT GTC AGC ACG GCA TAT CTA CAG ATC AGC AGC CTA AAG GCT 144  
 Asp Ser Ser Val Ser Thr Ala Tyr Leu Gln Ile Ser Ser Leu Lys Ala  
                   35                                  40                                  45

GAC GAC ACT GCA GTG TAT TAC TGT ACG AGA GAA GGG AAT ATG GAT GGT 192  
 Asp Asp Thr Ala Val Tyr Tyr Cys Thr Arg Glu Gly Asn Met Asp Gly  
                   50                                  55                                  60

TAC TTC CCT TTT ACT TAC TGG GGC CAG GGT ACC 225  
 Tyr Phe Pro Phe Thr Tyr Trp Gly Gln Gly Thr  
                   65                                  70                                  75

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 75 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Pro Gly Gln Gly Leu Lys Trp Met Gly Trp Ile Asn Thr Arg Asn Gly  
   1                                  5                                  10                                  15  
 Lys Ser Thr Tyr Val Asp Asp Phe Lys Gly Arg Phe Val Phe Ser Leu  
                   20                                  25                                  30  
 Asp Ser Ser Val Ser Thr Ala Tyr Leu Gln Ile Ser Ser Leu Lys Ala  
                   35                                  40                                  45  
 Asp Asp Thr Ala Val Tyr Tyr Cys Thr Arg Glu Gly Asn Met Asp Gly  
                   50                                  55                                  60  
 Tyr Phe Pro Phe Thr Tyr Trp Gly Gln Gly Thr  
                   65                                  70                                  75

(2) INFORMATION FOR SEQ ID NO:49:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

TTTCCTGGAC AAGGGCTCAA GTGGATG

27

## (2) INFORMATION FOR SEQ ID NO:50:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

TTTGGTACCC TGGCCCCAGT AAGT

24

## (2) INFORMATION FOR SEQ ID NO:51:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 363 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence

(B) LOCATION: 1...363

(D) OTHER INFORMATION: F9HZHC 1-1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

|                                                                 |     |
|-----------------------------------------------------------------|-----|
| CAG GTG CAA CTA GTG CAA TCT GGG TCT GAG TTG AAG AAG CCT GGG GCC | 48  |
| Gln Val Gln Leu Val Gln Ser Gly Ser Glu Leu Lys Lys Pro Gly Ala |     |
| 1 5 10 15                                                       |     |
| TCA GTG AAG GTT TCC TGC AAG GCC TCT GGA TAC ACC TTC ACT AAC TAT | 96  |
| Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr |     |
| 20 25 30                                                        |     |
| GGA ATG AAC TGG GTG CGA CAG GCC CCT GGA CAA GGG CTC AAG TGG ATG | 144 |
| Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Lys Trp Met |     |
| 35 40 45                                                        |     |
| GGA TGG ATA AAC ACC AGA AAT GGA AAG TCA ACA TAT GTT GAT GAC TTC | 192 |
| Gly Trp Ile Asn Thr Arg Asn Gly Lys Ser Thr Tyr Val Asp Asp Phe |     |
| 50 55 60                                                        |     |
| AAG GGA CGG TTT GTC TTC TCT CTA GAC TCC TCT GTC AGC ACG GCA TAT | 240 |
| Lys Gly Arg Phe Val Phe Ser Leu Asp Ser Ser Val Ser Thr Ala Tyr |     |
| 65 70 75 80                                                     |     |
| CTA CAG ATC AGC AGC CTA AAG GCT GAC GAC ACT GCA GTG TAT TAC TGT | 288 |
| Leu Gln Ile Ser Ser Leu Lys Ala Asp Asp Thr Ala Val Tyr Tyr Cys |     |
| 85 90 95                                                        |     |
| ACG AGA GAA GGG AAT ATG GAT GGT TAC TTC CCT TTT ACT TAC TGG GGC | 336 |
| Thr Arg Glu Gly Asn Met Asp Gly Tyr Phe Pro Phe Thr Tyr Trp Gly |     |
| 100 105 110                                                     |     |
| CAG GGT ACC CTG GTC ACC GTC TCC TCA                             | 363 |
| Gln Gly Thr Leu Val Thr Val Ser Ser                             |     |
| 115 120                                                         |     |

## (2) INFORMATION FOR SEQ ID NO:52:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 121 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (iii) HYPOTHETICAL: NO

## (iv) ANTISENSE: NO

## (v) FRAGMENT TYPE: internal

## (vi) ORIGINAL SOURCE:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Gln Val Gln Leu Val Gln Ser Gly Ser Glu Leu Lys Lys Pro Gly Ala  
 1 5 10 15  
 Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr  
 20 25 30  
 Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Lys Trp Met  
 35 40 45  
 Gly Trp Ile Asn Thr Arg Asn Gly Lys Ser Thr Tyr Val Asp Asp Phe  
 50 55 60  
 Lys Gly Arg Phe Val Phe Ser Leu Asp Ser Ser Val Ser Thr Ala Tyr  
 65 70 75 80  
 Leu Gln Ile Ser Ser Leu Lys Ala Asp Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95  
 Thr Arg Glu Gly Asn Met Asp Gly Tyr Phe Pro Phe Thr Tyr Trp Gly  
 100 105 110  
 Gln Gly Thr Leu Val Thr Val Ser Ser  
 115 120

## (2) INFORMATION FOR SEQ ID NO:53:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 82 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTISENSE: NO
- (v) FRAGMENT TYPE:
- (vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

|                                                                   |    |
|-------------------------------------------------------------------|----|
| CAACAGAGAC CTGGCCAGGC TCCCAAGCCC TGGATCTATG CCACGAGTAA CCTGGCTAGC | 60 |
| GGCGTCCCAG CCAGGTTCAG TG                                          | 82 |

(2) INFORMATION FOR SEQ ID NO:54:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 90 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTISENSE: NO
- (v) FRAGMENT TYPE:
- (vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

|                                                                  |    |
|------------------------------------------------------------------|----|
| GATCCACTGA ACCTGGCTGG GACGCCGCTA GCCAGGTAC TCGTGGCATA GATCCAGGGC | 60 |
| TTGGGAGCCT GGCCAGGTCT CTGTTGGTAC                                 | 90 |

(2) INFORMATION FOR SEQ ID NO:55:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 27 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: peptide
- (iii) HYPOTHETICAL: NO
- (iv) ANTISENSE: NO
- (v) FRAGMENT TYPE: internal
- (vi) ORIGINAL SOURCE:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Gln | Arg | Pro | Gly | Gln | Ala | Pro | Lys | Pro | Trp | Ile | Tyr | Ala | Thr | Ser |
| 1   |     |     |     | 5   |     |     |     | 10  |     |     |     |     |     | 15  |     |
| Asn | Leu | Ala | Ser | Gly | Val | Pro | Ala | Arg | Phe | Ser |     |     |     |     |     |
|     |     |     |     | 20  |     |     |     | 25  |     |     |     |     |     |     |     |

## (2) INFORMATION FOR SEQ ID NO:56:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 321 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (iii) HYPOTHETICAL: NO

## (iv) ANTISENSE: NO

## (v) FRAGMENT TYPE:

## (vi) ORIGINAL SOURCE:

## (ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 1...321
- (D) OTHER INFORMATION: F9HZLC 1-1

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| GAA | ATA | GTA | CTG | ACA | CAG | TCT | CCA | GCC | ACC | CTG | TCT | TTG | TCT | CCA | GGG | 48  |
| Glu | Ile | Val | Leu | Thr | Gln | Ser | Pro | Ala | Thr | Leu | Ser | Leu | Ser | Pro | Gly |     |
| 1   |     |     |     | 5   |     |     |     | 10  |     |     |     |     |     | 15  |     |     |
| GAA | AGA | GCC | ACC | CTC | TCC | TGC | AGG | GCC | AGC | TCA | AGT | GTA | AAT | TAC | ATG | 96  |
| Glu | Arg | Ala | Thr | Leu | Ser | Cys | Arg | Ala | Ser | Ser | Ser | Val | Asn | Tyr | Met |     |
|     |     |     |     | 20  |     |     |     | 25  |     |     |     |     |     | 30  |     |     |
| CAC | TGG | TAC | CAA | CAG | AGA | CCT | GGC | CAG | GCT | CCC | AAG | CCC | TGG | ATC | TAT | 144 |
| His | Trp | Tyr | Gln | Gln | Arg | Pro | Gly | Gln | Ala | Pro | Lys | Pro | Trp | Ile | Tyr |     |
|     |     |     |     | 35  |     |     |     | 40  |     |     |     |     |     | 45  |     |     |
| GCC | ACG | AGT | AAC | CTG | GCT | AGC | GGC | GTC | CCA | GCC | AGG | TTC | AGT | GGA | TCC | 192 |
| Ala | Thr | Ser | Asn | Leu | Ala | Ser | Gly | Val | Pro | Ala | Arg | Phe | Ser | Gly | Ser |     |
|     |     |     |     | 50  |     |     |     | 55  |     |     |     |     |     | 60  |     |     |



GGG TCT GGG ACA GAT TTC ACT CTC ACC ATC AGC AGT CTA GAG CCT GAA 240  
 Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu  
 65 70 75 80

GAT TTT GCG GTT TAT TAC TGT CAG CAG TGG AGT ATT AAC CCG CGG ACG 288  
 Asp Phe Ala Val Tyr Tyr Cys Gln Gln Trp Ser Ile Asn Pro Arg Thr  
 85 90 95

TTC GGC GGA GGG ACC AAG GTG GAG ATC AAA CGA 321  
 Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg  
 100 105

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 107 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly  
 1 5 10 15  
 Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Ser Ser Val Asn Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Arg Pro Gly Gln Ala Pro Lys Pro Trp Ile Tyr  
 35 40 45  
 Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser  
 50 55 60  
 Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu  
 65 70 75 80  
 Asp Phe Ala Val Tyr Tyr Cys Gln Gln Trp Ser Ile Asn Pro Arg Thr  
 85 90 95  
 Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg  
 100

(2) INFORMATION FOR SEQ ID NO:58:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 41 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

GATCCGGGTC TGGGACAGAT TACACTCTCA CGATATCCAG T

41

## (2) INFORMATION FOR SEQ ID NO:59:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 41 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

CTAGACTGGA TATCGTGAGA GTGTAATCTG TCCCAGACCC G

41

## (2) INFORMATION FOR SEQ ID NO:60:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO  
 (iv) ANTISENSE: NO  
 (v) FRAGMENT TYPE: internal  
 (vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser  
 1 5 10

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 321 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence  
 (B) LOCATION: 1...321  
 (D) OTHER INFORMATION: F9HZLC 1-2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

|                                                                 |     |
|-----------------------------------------------------------------|-----|
| GAA ATA GTA CTG ACA CAG TCT CCA GCC ACC CTG TCT TTG TCT CCA GGG | 48  |
| Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly |     |
| 1 5 10 15                                                       |     |
| GAA AGA GCC ACC CTC TCC TGC AGG GCC AGC TCA AGT GTA AAT TAC ATG | 96  |
| Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Ser Ser Val Asn Tyr Met |     |
| 20 25 30                                                        |     |
| CAC TGG TAC CAA CAG AGA CCT GGC CAG GCT CCC AAG CCC TGG ATC TAT | 144 |
| His Trp Tyr Gln Gln Arg Pro Gly Gln Ala Pro Lys Pro Trp Ile Tyr |     |
| 35 40 45                                                        |     |

GCC ACG AGT AAC CTG GCT AGC GGC GTC CCA GCC AGG TTC AGT GGA TCC 192  
 Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser  
 50 55 60

GGG TCT GGG ACA GAT TAC ACT CTC ACG ATA TCC AGT CTA GAG CCT GAA 240  
 Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu  
 65 70 75 80

GAT TTT GCG GTT TAT TAC TGT CAG CAG TGG AGT ATT AAC CCG CGG ACG 288  
 Asp Phe Ala Val Tyr Tyr Cys Gln Gln Trp Ser Ile Asn Pro Arg Thr  
 85 90 95

TTC GGC GGA GGG ACC AAG GTG GAG ATC AAA CGA 321  
 Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg  
 100 105

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 107 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly  
 1 5 10 15  
 Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Ser Ser Val Asn Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Arg Pro Gly Gln Ala Pro Lys Pro Trp Ile Tyr  
 35 40 45  
 Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser  
 50 55 60  
 Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu  
 65 70 75 80  
 Asp Phe Ala Val Tyr Tyr Cys Gln Gln Trp Ser Ile Asn Pro Arg Thr  
 85 90 95

[illegible]

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 165 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

```
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
```

|            |            |            |            |            |            |     |
|------------|------------|------------|------------|------------|------------|-----|
| AGTACTCACC | CAGAGCCCAA | GCAGCCTGAG | CGCCAGCGTG | GGTGACAGAG | TGACCATCAC | 60  |
| CTGCAGGGCC | AGCTCAAGTG | TAAATTACAT | GCACTGGTAC | CAGCAGAAGC | CAGGTAAGGC | 120 |
| TCCAAAGCCT | TGGATCTACG | CCACTAGTAA | CCTGGCTTCT | GGTGT      |            | 165 |

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 161 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

```
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
```

|            |            |            |            |            |            |     |
|------------|------------|------------|------------|------------|------------|-----|
| CCGCGGGTTA | ATACTCCACT | GCTGGCAGTA | GTAGGTGGCG | ATATCCTCTG | GCTGGAGGCT | 60  |
| GCTGATGGTG | AAGGTGTAGT | CTGTACCGCT | ACCGGATCCG | CTGAATCTGC | TTGGCACACC | 120 |
| AGAAGCCAGG | TTACTAGTGG | CGTAGATCCA | AGGCTTTGGA | G          |            | 161 |

105

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 280 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence

(B) LOCATION: 2...280

(D) OTHER INFORMATION:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

```

A GTA CTC ACC CAG AGC CCA AGC AGC CTG AGC GCC AGC GTG GGT GAC AGA 49
 Val Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg
 1 5 10 15

GTG ACC ATC ACC TGC AGG GCC AGC TCA AGT GTA AAT TAC ATG CAC TGG 97
 Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Asn Tyr Met His Trp
 20 25 30

TAC CAG CAG AAG CCA GGT AAG GCT CCA AAG CCT TGG ATC TAC GCC ACT 145
 Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Pro Trp Ile Tyr Ala Thr
 35 40 45

AGT AAC CTG GCT TCT GGT GTG CCA AGC AGA TTC AGC GGA TCC GGT AGC 193
 Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser
 50 55 60

GGT ACA GAC TAC ACC TTC ACC ATC AGC AGC CTC CAG CCA GAG GAT ATC 241
 Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile
 65 70 75 80

GCC ACC TAC TAC TGC CAG CAG TGG AGT ATT AAC CCG CGG 280
 Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ile Asn Pro Arg
 85 90

```

## (2) INFORMATION FOR SEQ ID NO:66:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 93 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (iii) HYPOTHETICAL: NO

## (iv) ANTISENSE: NO

## (v) FRAGMENT TYPE: internal

## (vi) ORIGINAL SOURCE:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

```

Val Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg
 1 5 10 15
Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Asn Tyr Met His Trp
 20 25 30
Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Pro Trp Ile Tyr Ala Thr
 35 40 45
Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser
 50 55 60
Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile
65 70 75 80
Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ile Asn Pro Arg
 85 90

```

## (2) INFORMATION FOR SEQ ID NO:67:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (iii) HYPOTHETICAL: NO

## (iv) ANTISENSE: NO

## (v) FRAGMENT TYPE:

## (vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

TTTAGTACTC ACCCAGAGCC CAAGCAG

27

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

TTCCGCGGGT TAATACTCCA CTGCTGG

27

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

CTCGAGCAGT ACTATCTGGG AGTGGACACC TGT

33

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 amino acids



- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: peptide
- (iii) HYPOTHETICAL: NO
- (iv) ANTISENSE: NO
- (v) FRAGMENT TYPE: N-terminal
- (vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Thr | Phe | Gly | Gln | Gly | Thr | Lys | Val | Glu | Ile | Lys | Arg | Thr | Val | Ala |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |
| Ala |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |

(2) INFORMATION FOR SEQ ID NO:71:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 48 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTISENSE: NO
- (v) FRAGMENT TYPE:
- (vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

GGACGTTTCGG CCAAGGGACC AAGGTGGAAA TCAAACGGAC TGTGGCGG

48

(2) INFORMATION FOR SEQ ID NO:72:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 52 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

52

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 321 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence  
(B) LOCATION: 1...321  
(D) OTHER INFORMATION: F9HZLC 2-0

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

CAG ATA GTA CTC ACC CAG AGC CCA AGC AGC CTG AGC GCC AGC GTG GGT 48  
Gln Ile Val Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
1 5 10 15

GAC AGA GTG ACC ATC ACC TGC AGG GCC AGC TCA AGT GTA AAT TAC ATG 96  
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Asn Tyr Met  
20 25 30

CAC TGG TAC CAG CAG AAG CCA GGT AAG GCT CCA AAG CCT TGG ATC TAC 144  
His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Pro Trp Ile Tyr  
35 40 45

GCC ACT AGT AAC CTG GCT TCT GGT GTG CCA AGC AGA TTC AGC GGA TCC 192  
Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser

50

55

60

GGT AGC GGT ACA GAC TAC ACC TTC ACC ATC AGC AGC CTC CAG CCA GAG 240  
 Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu  
 65 70 75 80

GAT ATC GCC ACC TAC TAC TGC CAG CAG TGG AGT ATT AAC CCG CGG ACG 288  
 Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ile Asn Pro Arg Thr  
 85 90 95

TTC GGC CAA GGG ACC AAG GTG GAA ATC AAA CGG 321  
 Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg  
 100 105

## (2) INFORMATION FOR SEQ ID NO:74:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 107 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (iii) HYPOTHETICAL: NO

## (iv) ANTISENSE: NO

## (v) FRAGMENT TYPE: internal

## (vi) ORIGINAL SOURCE:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

Gln Ile Val Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Asn Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Pro Trp Ile Tyr  
 35 40 45  
 Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60  
 Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu  
 65 70 75 80  
 Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ile Asn Pro Arg Thr  
 85 90 95  
 Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg  
 100 105

## (2) INFORMATION FOR SEQ ID NO:75:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 94 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (iii) HYPOTHETICAL: NO

## (iv) ANTISENSE: NO

## (v) FRAGMENT TYPE:

## (vi) ORIGINAL SOURCE:

## (ix) FEATURE:

(A) NAME/KEY: Coding Sequence

(B) LOCATION: 27...94

(D) OTHER INFORMATION:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

|                                                                  |    |
|------------------------------------------------------------------|----|
| GAATTCTGAG CACACAGGAC CTCACC ATG GGA TGG AGC TGT ATC ATC CTC TTC | 53 |
| Met Gly Trp Ser Cys Ile Ile Leu Phe                              |    |
| 1 5                                                              |    |
| TTG GTA GCA ACA GCT ACA GGT GTC CAC TCC CAG ATA GTA CT           | 94 |
| Leu Val Ala Thr Ala Thr Gly Val His Ser Gln Ile Val Leu          |    |
| 10 15 20                                                         |    |

## (2) INFORMATION FOR SEQ ID NO:76:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (iii) HYPOTHETICAL: NO

## (iv) ANTISENSE: NO

## (v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

```
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
 1 5 10 15
Val His Ser Gln Ile Val Leu
 20
```

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 401 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 27...401
- (D) OTHER INFORMATION: F9HZLC 1-3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

```
GAATTCTGAG CACACAGGAC CTCACC ATG GGA TGG AGC TGT ATC ATC CTC TTC 53
 Met Gly Trp Ser Cys Ile Ile Leu Phe
 1 5

TTG GTA GCA ACA GCT ACA GGT GTC CAC TCC CAG ATA GTA CTG ACA CAG 101
Leu Val Ala Thr Ala Thr Gly Val His Ser Gln Ile Val Leu Thr Gln
10 15 20 25

TCT CCA GCC ACC CTG TCT TTG TCT CCA GGG GAA AGA GCC ACC CTC TCC 149
Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser
 30 35 40

TGC AGG GCC AGC TCA AGT GTA AAT TAC ATG CAC TGG TAC CAA CAG AGA 197
```

Cys Arg Ala Ser Ser Ser Val Asn Tyr Met His Trp Tyr Gln Gln Arg  
 45 50 55  
 CCT GGC CAG GCT CCC AAG CCC TGG ATC TAT GCC ACG AGT AAC CTG GCT 245  
 Pro Gly Gln Ala Pro Lys Pro Trp Ile Tyr Ala Thr Ser Asn Leu Ala  
 60 65 70  
 AGC GGC GTC CCA GCC AGG TTC AGT GGA TCC GGG TCT GGG ACA GAT TAC 293  
 Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr  
 75 80 85  
 ACT CTC ACG ATA TCC AGT CTA GAG CCT GAA GAT TTT GCG GTT TAT TAC 341  
 Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr  
 90 95 100 105  
 TGT CAG CAG TGG AGT ATT AAC CCG CGG ACG TTC GGC GGA GGG ACC AAG 389  
 Cys Gln Gln Trp Ser Ile Asn Pro Arg Thr Phe Gly Gly Gly Thr Lys  
 110 115 120  
 GTG GAG ATC AAA 401  
 Val Glu Ile Lys  
 125

## (2) INFORMATION FOR SEQ ID NO:78:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 125 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (iii) HYPOTHETICAL: NO

## (iv) ANTISENSE: NO

## (v) FRAGMENT TYPE: internal

## (vi) ORIGINAL SOURCE:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly  
 1 5 10 15  
 Val His Ser Gln Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu  
 20 25 30  
 Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Ser Ser Val

|                                                                 |     |     |
|-----------------------------------------------------------------|-----|-----|
| 35                                                              | 40  | 45  |
| Asn Tyr Met His Trp Tyr Gln Gln Arg Pro Gly Gln Ala Pro Lys Pro |     |     |
| 50                                                              | 55  | 60  |
| Trp Ile Tyr Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe |     |     |
| 65                                                              | 70  | 75  |
| Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu |     |     |
| 85                                                              | 90  | 95  |
| Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Trp Ser Ile Asn |     |     |
| 100                                                             | 105 | 110 |
| Pro Arg Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys             |     |     |
| 115                                                             | 120 | 125 |

## (2) INFORMATION FOR SEQ ID NO:79:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 81 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (iii) HYPOTHETICAL: NO

## (iv) ANTISENSE: NO

## (v) FRAGMENT TYPE:

## (vi) ORIGINAL SOURCE:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

|                                                                   |    |
|-------------------------------------------------------------------|----|
| AGGCCTCTGG ATACACCTTC ACTAACTATG GAATGAACTG GGTGCGACAG GCCCCTGGAC | 60 |
| AAGGGCTCGA GTGGATGGGA T                                           | 81 |

## (2) INFORMATION FOR SEQ ID NO:80:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 99 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (iii) HYPOTHETICAL: NO

## (iv) ANTISENSE: NO

## (v) FRAGMENT TYPE:

## (vi) ORIGINAL SOURCE:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

|                                                                   |    |
|-------------------------------------------------------------------|----|
| TGTCTAGAGA GAAGACAAAC CGTCCCTTGA AGTCATCAAC ATATGTTGAC TTTCCATTTC | 60 |
| TGGTGTTTAT CCATCCCATC CACTCGAGCC CTTGTCCAG                        | 99 |

## (2) INFORMATION FOR SEQ ID NO:81:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 87 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

|                                                                   |    |
|-------------------------------------------------------------------|----|
| GGTTTGTCTT CTCTCTAGAC ACCTCTGTCA GCACGGCATA TCTACAGATC AGCAGCCTAA | 60 |
| AGGCTGAGGA CACTGCAGTG TATTTCT                                     | 87 |

## (2) INFORMATION FOR SEQ ID NO:82:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

|                                                                 |    |
|-----------------------------------------------------------------|----|
| GGTACCCTGG CCCAGTAAG TAAAAGGGAA GTAACCATCC ATATTCCTT CTCTCGTACA | 60 |
| GAAATACACT GCAGTGTCTT CAGCCT                                    | 86 |



## (2) INFORMATION FOR SEQ ID NO:83:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 278 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (iii) HYPOTHETICAL: NO

## (iv) ANTISENSE: NO

## (v) FRAGMENT TYPE:

## (vi) ORIGINAL SOURCE:

## (ix) FEATURE:

## (A) NAME/KEY: Coding Sequence

## (B) LOCATION: 3...278

## (D) OTHER INFORMATION:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

```

AG GCC TCT GGA TAC ACC TTC ACT AAC TAT GGA ATG AAC TGG GTG CGA 47
 Ala Ser Gly Tyr Thr Phe Thr Asn Tyr Gly Met Asn Trp Val Arg
 1 5 10 15

CAG GCC CCT GGA CAA GGG CTC GAG TGG ATG GGA TGG ATA AAC ACC AGA 95
 Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Thr Arg
 20 25 30

AAT GGA AAG TCA ACA TAT GTT GAT GAC TTC AAG GGA CGG TTT GTC TTC 143
 Asn Gly Lys Ser Thr Tyr Val Asp Asp Phe Lys Gly Arg Phe Val Phe
 35 40 45

TCT CTA GAC ACC TCT GTC AGC ACG GCA TAT CTA CAG ATC AGC AGC CTA 191
 Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr Leu Gln Ile Ser Ser Leu
 50 55 60

AAG GCT GAG GAC ACT GCA GTG TAT TTC TGT ACG AGA GAA GGG AAT ATG 239
 Lys Ala Glu Asp Thr Ala Val Tyr Phe Cys Thr Arg Glu Gly Asn Met
 65 70 75

GAT GGT TAC TTC CCT TTT ACT TAC TGG GGC CAG GGT ACC 278
 Asp Gly Tyr Phe Pro Phe Thr Tyr Trp Gly Gln Gly Thr

```

80

85

90

## (2) INFORMATION FOR SEQ ID NO:84:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 92 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (iii) HYPOTHETICAL: NO

## (iv) ANTISENSE: NO

## (v) FRAGMENT TYPE: internal

## (vi) ORIGINAL SOURCE:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

```

Ala Ser Gly Tyr Thr Phe Thr Asn Tyr Gly Met Asn Trp Val Arg Gln
 1 5 10 15
Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Thr Arg Asn
 20 25 30
Gly Lys Ser Thr Tyr Val Asp Asp Phe Lys Gly Arg Phe Val Phe Ser
 35 40 45
Leu Asp Thr Ser Val Ser Thr Ala Tyr Leu Gln Ile Ser Ser Leu Lys
 50 55 60
Ala Glu Asp Thr Ala Val Tyr Phe Cys Thr Arg Glu Gly Asn Met Asp
65 70 75 80
Gly Tyr Phe Pro Phe Thr Tyr Trp Gly Gln Gly Thr
 85 90

```

## (2) INFORMATION FOR SEQ ID NO:85:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (iii) HYPOTHETICAL: NO

## (iv) ANTISENSE: NO

## (v) FRAGMENT TYPE:

## (vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

AGGCCTCTGG ATACACCTTC ACTAACTATG

30

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

GGTACCCTGG CCCCAGTAAG TAAAAG

26

(2) INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

CCAGACTCGA CTAGTTGGAT CTGGGAGTGG ACACCTG

37

(2) INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 446 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA  
 (iii) HYPOTHETICAL: NO  
 (iv) ANTISENSE: NO  
 (v) FRAGMENT TYPE:  
 (vi) ORIGINAL SOURCE:  
 (ix) FEATURE:

(A) NAME/KEY: Coding Sequence  
 (B) LOCATION: 27...446  
 (D) OTHER INFORMATION: F9HZHC 3-0

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

|                                                                  |     |
|------------------------------------------------------------------|-----|
| GAATTCTGAG CACACAGGAC CTCACC ATG GGA TGG AGC TGT ATC ATC CTC TTC | 53  |
| Met Gly Trp Ser Cys Ile Ile Leu Phe                              |     |
| 1 5                                                              |     |
| TTG GTA GCA ACA GCT ACA GGT GTC CAC TCC CAG ATC CAA CTA GTG CAA  | 101 |
| Leu Val Ala Thr Ala Thr Gly Val His Ser Gln Ile Gln Leu Val Gln  |     |
| 10 15 20 25                                                      |     |
| TCT GGG TCT GAG TTG AAG AAG CCT GGG GCC TCA GTG AAG GTT TCC TGC  | 149 |
| Ser Gly Ser Glu Leu Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys  |     |
| 30 35 40                                                         |     |
| AAG GCC TCT GGA TAC ACC TTC ACT AAC TAT GGA ATG AAC TGG GTG CGA  | 197 |
| Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr Gly Met Asn Trp Val Arg  |     |
| 45 50 55                                                         |     |
| CAG GCC CCT GGA CAA GGG CTC GAG TGG ATG GGA TGG ATA AAC ACC AGA  | 245 |
| Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Thr Arg  |     |
| 60 65 70                                                         |     |
| AAT GGA AAG TCA ACA TAT GTT GAT GAC TTC AAG GGA CGG TTT GTC TTC  | 293 |
| Asn Gly Lys Ser Thr Tyr Val Asp Asp Phe Lys Gly Arg Phe Val Phe  |     |
| 75 80 85                                                         |     |
| TCT CTA GAC ACC TCT GTC AGC ACG GCA TAT CTA CAG ATC AGC AGC CTA  | 341 |

Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr Leu Gln Ile Ser Ser Leu  
 90 95 100 105

AAG GCT GAG GAC ACT GCA GTG TAT TTC TGT ACG AGA GAA GGG AAT ATG 389  
 Lys Ala Glu Asp Thr Ala Val Tyr Phe Cys Thr Arg Glu Gly Asn Met  
 110 115 120

GAT GGT TAC TTC CCT TTT ACT TAC TGG GGC CAG GGT ACC CTG GTC ACC 437  
 Asp Gly Tyr Phe Pro Phe Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr  
 125 130 135

GTC TCC TCT 446  
 Val Ser Ser  
 140

(2) INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 140 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly  
 1 5 10 15  
 Val His Ser Gln Ile Gln Leu Val Gln Ser Gly Ser Glu Leu Lys Lys  
 20 25 30  
 Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe  
 35 40 45  
 Thr Asn Tyr Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu  
 50 55 60  
 Glu Trp Met Gly Trp Ile Asn Thr Arg Asn Gly Lys Ser Thr Tyr Val  
 65 70 75 80  
 Asp Asp Phe Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser  
 85 90 95  
 Thr Ala Tyr Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|     | 100 |     | 105 |     | 110 |     |     |     |     |     |     |     |     |     |     |
| Tyr | Phe | Cys | Thr | Arg | Glu | Gly | Asn | Met | Asp | Gly | Tyr | Phe | Pro | Phe | Thr |
|     | 115 |     | 120 |     | 125 |     |     |     |     |     |     |     |     |     |     |
| Tyr | Trp | Gly | Gln | Gly | Thr | Leu | Val | Thr | Val | Ser | Ser |     |     |     |     |
|     | 130 |     | 135 |     | 140 |     |     |     |     |     |     |     |     |     |     |

## (2) INFORMATION FOR SEQ ID NO:90:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 90 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

|                                                                  |    |
|------------------------------------------------------------------|----|
| AGTACTGACA CAGTCTCCAT CCTCCCTGTC TGCATCTGTT GGGACAGAG TCACCATCAC | 60 |
| TTGCAGGGCC AGCTCAAGTG TAAATTACAT                                 | 90 |

## (2) INFORMATION FOR SEQ ID NO:91:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 108 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

|                                                                   |     |
|-------------------------------------------------------------------|-----|
| CTTGATGGGA CGCCGCTAGC CAGGTTACTC GTGGCATAGA TCCAGGGCTT GGGAGCTTTG | 60  |
| CCAGGTTTCT GTTGGTACCA GTGCATGTAA TTACACTTG AGCTGGCC               | 108 |

## (2) INFORMATION FOR SEQ ID NO:92:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 108 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

|                                                                   |     |
|-------------------------------------------------------------------|-----|
| TAACCTGGCT AGCGGCGTCC CATCAAGGTT CAGTGGATCC GGGTCTGGGA CAGATTACAC | 60  |
| TCTCACCATA TCCAGTCTAC AACCTGAAGA TTTTGCGACT TATTACTG              | 108 |

## (2) INFORMATION FOR SEQ ID NO:93:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

|                                                                   |     |
|-------------------------------------------------------------------|-----|
| GGCGCCGCCA CAGTTCGTTT GATCTCCAGC TTGGTCCCTC CGCCGAACGT CCGCGGGTTA | 60  |
| ATACTCCACT GCTGACAGTA ATAAGTCGCA AAATCTTCAG GT                    | 102 |

## (2) INFORMATION FOR SEQ ID NO:94:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 330 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence

(B) LOCATION: 2...328

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

```

A GTA CTG ACA CAG TCT CCA TCC TCC CTG TCT GCA TCT GTT GGG GAC AGA 49
 Val Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg
 1 5 10 15

GTC ACC ATC ACT TGC AGG GCC AGC TCA AGT GTA AAT TAC ATG CAC TGG 97
 Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Asn Tyr Met His Trp
 20 25 30

TAC CAA CAG AAA CCT GGC AAA GCT CCC AAG CCC TGG ATC TAT GCC ACG 145
 Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Pro Trp Ile Tyr Ala Thr
 35 40 45

AGT AAC CTG GCT AGC GGC GTC CCA TCA AGG TTC AGT GGA TCC GGG TCT 193
 Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser
 50 55 60

GGG ACA GAT TAC ACT CTC ACG ATA TCC AGT CTA CAA CCT GAA GAT TTT 241
 Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe
 65 70 75 80

GCG ACT TAT TAC TGT CAG CAG TGG AGT ATT AAC CCG CGG ACG TTC GGC 289
 Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ile Asn Pro Arg Thr Phe Gly
 85 90 95

GGA GGG ACC AAG CTG GAG ATC AAA CGA ACT GTG GCG GCG CC 330
 Gly Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala
 100 105

```



## (2) INFORMATION FOR SEQ ID NO:95:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 109 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (iii) HYPOTHETICAL: NO

## (iv) ANTISENSE: NO

## (v) FRAGMENT TYPE: internal

## (vi) ORIGINAL SOURCE:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |    |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|----|
| Val | Leu | Thr | Gln | Ser | Pro | Ser | Ser | Leu | Ser | Ala | Ser | Val | Gly | Asp | Arg | 1   | 5   | 10 | 15 |
| Val | Thr | Ile | Thr | Cys | Arg | Ala | Ser | Ser | Ser | Val | Asn | Tyr | Met | His | Trp | 20  | 25  | 30 |    |
| Tyr | Gln | Gln | Lys | Pro | Gly | Lys | Ala | Pro | Lys | Pro | Trp | Ile | Tyr | Ala | Thr | 35  | 40  | 45 |    |
| Ser | Asn | Leu | Ala | Ser | Gly | Val | Pro | Ser | Arg | Phe | Ser | Gly | Ser | Gly | Ser | 50  | 55  | 60 |    |
| Gly | Thr | Asp | Tyr | Thr | Leu | Thr | Ile | Ser | Ser | Leu | Gln | Pro | Glu | Asp | Phe | 65  | 70  | 75 | 80 |
| Ala | Thr | Tyr | Tyr | Cys | Gln | Gln | Trp | Ser | Ile | Asn | Pro | Arg | Thr | Phe | Gly | 85  | 90  | 95 |    |
| Gly | Gly | Thr | Lys | Leu | Glu | Ile | Lys | Arg | Thr | Val | Ala | Ala |     |     |     | 100 | 105 |    |    |

## (2) INFORMATION FOR SEQ ID NO:96:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (iii) HYPOTHETICAL: NO

## (iv) ANTISENSE: NO

## (v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

CAAGTACTGA CACAGTCTCC ATCCTC

26

(2) INFORMATION FOR SEQ ID NO:97:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

AGGGCGCCGC CACAGTTCGT TTGATC

26

(2) INFORMATION FOR SEQ ID NO:98:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 412 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence

(B) LOCATION: 27...412

(D) OTHER INFORMATION: F9HZLC 3-0

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

GAATTCTGAG CACACAGGAC CTCACC ATG GGA TGG AGC TGT ATC ATC CTC TTC 53  
Met Gly Trp Ser Cys Ile Ile Leu Phe  
1 5

TTG GTA GCA ACA GCT ACA GGT GTC CAC TCC CAG ATA GTA CTG ACA CAG 101  
Leu Val Ala Thr Ala Thr Gly Val His Ser Gln Ile Val Leu Thr Gln  
10 15 20 25

TCT CCA TCC TCC CTG TCT GCA TCT GTT GGG GAC AGA GTC ACC ATC ACT 149  
Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr  
30 35 40

TGC AGG GCC AGC TCA AGT GTA AAT TAC ATG CAC TGG TAC CAA CAG AAA 197  
Cys Arg Ala Ser Ser Ser Val Asn Tyr Met His Trp Tyr Gln Gln Lys  
45 50 55

CCT GGC AAA GCT CCC AAG CCC TGG ATC TAT GCC ACG AGT AAC CTG GCT 245  
Pro Gly Lys Ala Pro Lys Pro Trp Ile Tyr Ala Thr Ser Asn Leu Ala  
60 65 70

AGC GGC GTC CCA TCA AGG TTC AGT GGA TCC GGG TCT GGG ACA GAT TAC 293  
Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr  
75 80 85

ACT CTC ACG ATA TCC AGT CTA CAA CCT GAA GAT TTT GCG ACT TAT TAC 341  
Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr  
90 95 100 105

TGT CAG CAG TGG AGT ATT AAC CCG CGG ACG TTC GGC GGA GGG ACC AAG 389  
Cys Gln Gln Trp Ser Ile Asn Pro Arg Thr Phe Gly Gly Gly Thr Lys  
110 115 120

CTG GAG ATC AAA CGA ACT GTG GC 412  
Leu Glu Ile Lys Arg Thr Val Val  
125

## (2) INFORMATION FOR SEQ ID NO:99:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 129 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

```

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
 1 5 10 15
Val His Ser Gln Ile Val Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala
 20 25 30
Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val
 35 40 45
Asn Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Pro
 50 55 60
Trp Ile Tyr Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe
 65 70 75 80
Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu
 85 90 95
Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ile Asn
 100 105 110
Pro Arg Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Thr Val
 115 120 125
Val

```

(2) INFORMATION FOR SEQ ID NO:100:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

CAAATAGTAC TCTCCAGTC TCCAGC

26

## (2) INFORMATION FOR SEQ ID NO:101:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 41 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

GGATAAGCTT GCGCCGCAA CAGTCGGTTT GATTTCAGC T

41

## (2) INFORMATION FOR SEQ ID NO:102:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 335 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence

(B) LOCATION: 1...335

(D) OTHER INFORMATION:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

CAG ATA GTA CTC TCC CAG TCT CCA GCA ATC CTG TCT GCA TCT CCA GGG  
 Gln Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly

48

| 1                                                               | 5   | 10  | 15 |     |
|-----------------------------------------------------------------|-----|-----|----|-----|
| GAG AAG GTC ACA ATG ACT TGC AGG GCC AGC TCA AGT GTA AAT TAC ATG |     |     |    | 96  |
| Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Asn Tyr Met |     |     |    |     |
| 20                                                              | 25  | 30  |    |     |
| CAC TGG TAC CAG CAG AAG CCA GGA TCC TCC CCC AAA CCC TGG ATT TAT |     |     |    | 144 |
| His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Tyr |     |     |    |     |
| 35                                                              | 40  | 45  |    |     |
| GCC ACA TCC AAC CTG GCT TCT GGA GTC CCT GCT CGC TTC AGT GGC AGT |     |     |    | 192 |
| Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser |     |     |    |     |
| 50                                                              | 55  | 60  |    |     |
| GGG TCT GGG ACC TCT TAC TCT CTC ACA ATC AGC AGA GTG GAG GCT GAA |     |     |    | 240 |
| Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Val Glu Ala Glu |     |     |    |     |
| 65                                                              | 70  | 75  | 80 |     |
| GAT GCT GCC ACT TAT TAC TGC CAG CAG TGG AGT ATT AAC CCA CGG ACG |     |     |    | 288 |
| Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ile Asn Pro Arg Thr |     |     |    |     |
| 85                                                              | 90  | 95  |    |     |
| TTC GGT GGA GGC ACC AAG CTG GAA ATC AAA CGG ACT GTT GCG GCG CC  |     |     |    | 335 |
| Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro |     |     |    |     |
| 100                                                             | 105 | 110 |    |     |

## (2) INFORMATION FOR SEQ ID NO:103:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 112 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (iii) HYPOTHETICAL: NO

## (iv) ANTISENSE: NO

## (v) FRAGMENT TYPE: internal

## (vi) ORIGINAL SOURCE:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

| 1                                                               | 5 | 10 | 15 |
|-----------------------------------------------------------------|---|----|----|
| Gln Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly |   |    |    |

Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Asn Tyr Met  
                   20                                  25                                  30  
 His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Tyr  
                   35                                  40                                  45  
 Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser  
                   50                                  55                                  60  
 Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Val Glu Ala Glu  
 65                                  70                                  75                                  80  
 Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ile Asn Pro Arg Thr  
                                   85                                  90                                  95  
 Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro  
                   100                                  105                                  110

## (2) INFORMATION FOR SEQ ID NO:104:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 318 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (iii) HYPOTHETICAL: NO

## (iv) ANTISENSE: NO

## (v) FRAGMENT TYPE:

## (vi) ORIGINAL SOURCE:

## (ix) FEATURE:

## (A) NAME/KEY: Coding Sequence

## (B) LOCATION: 1...318

## (D) OTHER INFORMATION:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

CAG ATA GTA CTC TCC CAG TCT CCA GCA ATC CTG TCT GCA TCT CCA GGG 48  
 Gln Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly  
 1                                  5                                  10                                  15  
  
 GAG AAG GTC ACA ATG ACT TGC AGG GCC AGC TCA AGT GTA AAT TAC ATG 96  
 Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Asn Tyr Met  
                   20                                  25                                  30  
  
 CAC TGG TAC CAG CAG AAG CCA GGA TCC TCC CCC AAA CCC TGG ATT TAT 144

```

His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Tyr
 35 40 45

GCC ACA TCC AAC CTG GCT TCT GGA GTC CCT GCT CGC TTC AGT GGC AGT 192
Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
 50 55 60

GGG TCT GGG ACC TCT TAC TCT CTC ACA ATC AGC AGA GTG GAG GCT GAA 240
Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Val Glu Ala Glu
 65 70 75 80

GAT GCT GCC ACT TAT TAC TGC CAG CAG TGG AGT ATT AAC CCA CGG ACG 288
Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ile Asn Pro Arg Thr
 85 90 95

TTC GGT GGA GGC ACC AAG CTG GAA ATC AAA 318
Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
 100 105

```

## (2) INFORMATION FOR SEQ ID NO:105:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 106 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (iii) HYPOTHETICAL: NO

## (iv) ANTISENSE: NO

## (v) FRAGMENT TYPE: internal

## (vi) ORIGINAL SOURCE:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:

```

Gln Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly
 1 5 10 15
Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Asn Tyr Met
 20 25 30
His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Tyr
 35 40 45
Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
 50 55 60
Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Val Glu Ala Glu

```



|     |     |     |     |
|-----|-----|-----|-----|
| 65  | 70  | 75  | 80  |
| Asp | Ala | Ala | Thr |
| Tyr | Tyr | Cys | Gln |
| Gln | Trp | Ser | Ile |
| Asn | Pro | Arg | Thr |
| 85  | 90  | 95  |     |
| Phe | Gly | Gly | Gly |
| Thr | Lys | Leu | Glu |
| Ile | Lys |     |     |
| 100 | 105 |     |     |

## (2) INFORMATION FOR SEQ ID NO:106:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:

CAGATCCAAC TAGTGCAGTC TGGACCTGAG

30

## (2) INFORMATION FOR SEQ ID NO:107:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:

TTAAGCTTGC TAGCTGCAGA GACAGTGACC AG

32

## (2) INFORMATION FOR SEQ ID NO:108:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 369 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence

(B) LOCATION: 1...369

(D) OTHER INFORMATION:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:108:

|                                                                 |     |
|-----------------------------------------------------------------|-----|
| CAG ATC CAA CTA GTG CAG TCT GGA CCT GAG CTG AAG AAG CCT GGA GAG | 48  |
| Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu |     |
| 1 5 10 15                                                       |     |
| ACA GTC AAG ATC TCC TGC AAG GCT TCT GGG TAC ACC TTC ACA AAC TAT | 96  |
| Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr |     |
| 20 25 30                                                        |     |
| GGA ATG AAC TGG GTG AAG CAG GCT CCA GGA AAG GGT TTA AAG TGG ATG | 144 |
| Gly Met Asn Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp Met |     |
| 35 40 45                                                        |     |
| GGC TGG ATA AAC ACC AGA AAT GGA AAG TCA ACA TAT GTT GAT GAC TTC | 192 |
| Gly Trp Ile Asn Thr Arg Asn Gly Lys Ser Thr Tyr Val Asp Asp Phe |     |
| 50 55 60                                                        |     |
| AAG GGA CGG TTT GCC TTC TCT TTG GAA AGC TCT GCC AGC ACT GCC AAT | 240 |
| Lys Gly Arg Phe Ala Phe Ser Leu Glu Ser Ser Ala Ser Thr Ala Asn |     |
| 65 70 75 80                                                     |     |
| TTG CAG ATC GAC AAC CTC AAA GAT GAG GAC ACG GCT ACA TAT TTC TGT | 288 |
| Leu Gln Ile Asp Asn Leu Lys Asp Glu Asp Thr Ala Thr Tyr Phe Cys |     |
| 85 90 95                                                        |     |

CAA GGG ACT CTG GTC ACT GTC TCT GCA GCT AGC 369  
Gln Gly Thr Leu Val Thr Val Ser Ala Ala Ser  
115 120

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 123 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

- ```
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
```

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:109:

Gln	Ile	Gln	Leu	Val	Gln	Ser	Gly	Pro	Glu	Leu	Lys	Lys	Pro	Gly	Glu
1				5					10					15	
Thr	Val	Lys	Ile	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Asn	Tyr
			20					25					30		
Gly	Met	Asn	Trp	Val	Lys	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Lys	Trp	Met
		35					40					45			
Gly	Trp	Ile	Asn	Thr	Arg	Asn	Gly	Lys	Ser	Thr	Tyr	Val	Asp	Asp	Phe
	50					55				60					
Lys	Gly	Arg	Phe	Ala	Phe	Ser	Leu	Glu	Ser	Ser	Ala	Ser	Thr	Ala	Asn
65					70					75					80
Leu	Gln	Ile	Asp	Asn	Leu	Lys	Asp	Glu	Asp	Thr	Ala	Thr	Tyr	Phe	Cys
			85						90					95	
Thr	Arg	Glu	Gly	Asn	Met	Asp	Gly	Tyr	Phe	Pro	Phe	Thr	Tyr	Trp	Gly
			100					105					110		
Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ala	Ala	Ser					
		115					120								

(2) INFORMATION FOR SEQ ID NO:110:

- (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 363 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
 (iii) HYPOTHETICAL: NO
 (iv) ANTISENSE: NO
 (v) FRAGMENT TYPE:
 (vi) ORIGINAL SOURCE:
 (ix) FEATURE:

(A) NAME/KEY: Coding Sequence
 (B) LOCATION: 1...363
 (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:110:

CAG ATC CAA CTA GTG CAG TCT GGA CCT GAG CTG AAG AAG CCT GGA GAG	48
Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu	
1 5 10 15	
ACA GTC AAG ATC TCC TGC AAG GCT TCT GGG TAC ACC TTC ACA AAC TAT	96
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr	
20 25 30	
GGA ATG AAC TGG GTG AAG CAG GCT CCA GGA AAG GGT TTA AAG TGG ATG	144
Gly Met Asn Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp Met	
35 40 45	
GGC TGG ATA AAC ACC AGA AAT GGA AAG TCA ACA TAT GTT GAT GAC TTC	192
Gly Trp Ile Asn Thr Arg Asn Gly Lys Ser Thr Tyr Val Asp Asp Phe	
50 55 60	
AAG GGA CGG TTT GCC TTC TCT TTG GAA AGC TCT GCC AGC ACT GCC AAT	240
Lys Gly Arg Phe Ala Phe Ser Leu Glu Ser Ser Ala Ser Thr Ala Asn	
65 70 75 80	
TTG CAG ATC GAC AAC CTC AAA GAT GAG GAC ACG GCT ACA TAT TTC TGT	288
Leu Gln Ile Asp Asn Leu Lys Asp Glu Asp Thr Ala Thr Tyr Phe Cys	
85 90 95	
ACA AGA GAA GGG AAT ATG GAT GGT TAC TTC CCT TTT ACT TAC TGG GGC	336

Thr Arg Glu Gly Asn Met Asp Gly Tyr Phe Pro Phe Thr Tyr Trp Gly
 100 105 110

CAA GGG ACT CTG GTC ACT GTC TCT GCA
 Gln Gly Thr Leu Val Thr Val Ser Ala
 115 120

363

(2) INFORMATION FOR SEQ ID NO:111:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 121 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:111:

Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu
 1 5 10 15
 Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
 20 25 30
 Gly Met Asn Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp Met
 35 40 45
 Gly Trp Ile Asn Thr Arg Asn Gly Lys Ser Thr Tyr Val Asp Asp Phe
 50 55 60
 Lys Gly Arg Phe Ala Phe Ser Leu Glu Ser Ser Ala Ser Thr Ala Asn
 65 70 75 80
 Leu Gln Ile Asp Asn Leu Lys Asp Glu Asp Thr Ala Thr Tyr Phe Cys
 85 90 95
 Thr Arg Glu Gly Asn Met Asp Gly Tyr Phe Pro Phe Thr Tyr Trp Gly
 100 105 110
 Gln Gly Thr Leu Val Thr Val Ser Ala
 115 120